JOURNAL OF THE NATIONAL CANCER INSTITUTE

December 1950
Volume 11
Number 3

NATIONAL INSTITUTES OF HEALTH • U. S. PUBLIC HEALTH SERVICE

ON THE MECHANISM OF CANCER INDUCTION BY ULTRAVIOLET RADIATION [1]

HAROLD F. BLUM,[2] *National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and Department of Biology, Princeton University,*[3] *New Jersey*

I. INTRODUCTION
II. EXPERIMENTAL DATA
1. Animal Material
2. Development Time
3. Dosage of Ultraviolet Radiation
4. Summary of Data
III. THEORETICAL
A. RELATIVE GROWTH
B. THEORY OF PROGRESSIVE ACCELERATION OF TUMOR GROWTH
1. Basic Model
2. Development Time
3. Rate of Growth
4. Maximum Limit of Growth Rate
5. Effect of Interruption of Dosage Schedule
6. Summing Up
C. ALTERNATIVE THEORIES
1. Separable Induction and Growth
2. Somatic Mutations
D. OTHER CARCINOGENS
IV. SUMMARY AND CONCLUSIONS
V. ACKNOWLEDGMENTS

I. INTRODUCTION

This paper describes a tentative theory regarding carcinogenesis, introduced to explain data on the induction of cancer by ultraviolet radiation. By assuming that successive doses of radiation progressively accelerate the relative rate of cell proliferation, each dose bringing about an increase in rate, the theory achieves good quantitative agreement with

[1] Received for publication June 20, 1950.
[2] Prepared, in part, while a Guggenheim Fellow, at the Marine Biological Laboratory, Woods Hole, Mass.
[3] Present address.

922686—51——1

the experimental data. Other currently accepted theories fail when examined quantitatively to explain these data satisfactorily. Thus, the concept herein presented provides a reasonable, and apparently unique, rationalization of the experimental findings to date. It is possible, of course, that further evidence may require revision of this concept, but since the principles upon which the treatment is based involve ideas fundamental to any adequate explanation of the mechanism of carcinogenesis, the paper should have heuristic value no matter what the ultimate fate of the theory itself.

A basic difficulty in the study of cancer at the present time is that it is only possible to detect a tumor after it has reached a relatively advanced stage. One does not recognize a cancer cell as such, but only in company with other cancer cells; and, hence, cannot know when a cancer first arises nor follow quantitatively the earliest stages of its development. Thus, any theory of cancer induction is based on an extrapolation, the present treatment having the merit that it is a quantitative extrapolation as contrasted to the purely qualitative ones upon which most theories of carcinogenesis are based.

II. EXPERIMENTAL DATA[4]

The data used herein come from experiments on the induction of cancer by ultraviolet radiation. These experiments—terminated as a result of the war—were conducted with considerable care from a quantitative point of view; and, because of limitations inherent in the biological material itself, it is improbable that much better measurements of the kind are obtainable. One would like to have more data regarding certain points, but it is unlikely that they would support a more definite conclusion, which probably must be sought with other techniques. A brief general survey of these data, which have been published in a series of separate papers in this journal (*2–6, 11, 12, 14, 20, 21, 23*), will be presented before entering upon the theoretical analysis.

II. 1. Animal Material

The experimental animals were albino mice, strain A, all of male sex, and all of approximately the same age at the start of a given experiment. With this genetically homogeneous material, and uniform diet and treatment, biological variability should have been reduced to a minimum.

II. 2. Development Time

These mice were subjected to carefully measured doses of ultraviolet radiation which were given at regular intervals. After a considerable number of doses tumors appeared on the ears of the mice; the time of appearance depending upon the size and frequency of the dose. The time from the application of the first dose to the appearance of such a tumor in

[4] The original data have been rechecked in preparing this paper. A few minor corrections have been made which slightly alter values published earlier. All these changes are, however, of trivial magnitude.

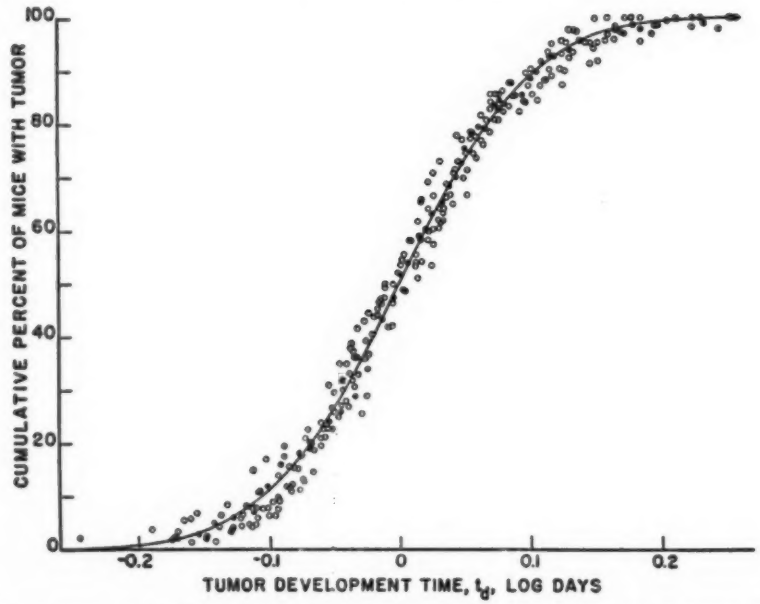
a given mouse was taken as the "development"[5] time, and is symbolized herein as t_d. For practical reasons the "appearance of tumor" was taken as the time at which a tumor of a given estimated size—approximately 100 mm^3 in volume and consisting of about 10^8 cells—was present on the ear. At this time the tumors were doubling their volume every few days, and hence the error in the estimation of t_d was relatively small (see sec. III. B. 3). Histopathological examination showed that the tumors were malignant and predominantly sarcomas (*20*, *21*). That our criterion of tumor appearance was adequately accurate is indicated by the reproducibility of the experiments (*5*, *11*).

The variance of t_d within identically treated populations of mice is relatively great, but less than that found with other types of carcinogenesis. When the same dose of radiation was applied at regular intervals until the tumor appeared, the distribution of t_d in such a population followed a smooth quantitative relationship, which may be described as follows. If the cumulative percent of mice with tumors is plotted against the logarithm of t_d, as in text-figure 1, the integral of a normal distribution curve is obtained. The position of this curve on the time axis is shifted with dose and schedule of exposure, as is indicated diagrammatically in text-figure 2. In text-figure 1 data from a number of experiments involving different dosages are brought together to a common point at 50 percent tumor incidence; the drawn curve represents an "average" for all the groups concerned (standard deviation=0.081 log days). The uniform distribution of the points around the curve indicates the reliability of the data, the constancy of the slope and shape of the curve; and, hence the validity of the relationship indicated in text-figure 2. The success of the *log* t_d plotting is shown by comparison of text-figure 2 with text-figure 3, t_d being plotted as the abscissa in the latter instead of *log* t_d. Change in the schedule of doses, *e. g.*, by discontinuing them after a given time, markedly alters the shape of the curves as is evident in text-figures 8 and 9. Repeated dosage was necessary to induce tumors; in no case did a single dose—no matter how great—result in the appearance of a tumor (*4*).

II. 3. Dosage of Ultraviolet Radiation

The wavelengths of ultraviolet radiation which are effective in inducing tumors are those shorter than approximately 0.32 μ; the experimental evidence establishing this wavelength limit has been reviewed recently in this journal (*7*). The long wavelength limit is the same as that for sunburn and destructive effects in living cells in general; but the physical nature of the system renders it infeasible with available methods accurately to identify the light absorber by comparison of an action spectrum with absorption spectra (*2*, *9*). For convenience, dosage is indicated, herein, in "energy units," each unit having the value 10^7 ergs cm^{-2} of radiation of wavelengths 0.313 μ and shorter, delivered by a particular type of mercury arc [for details of the measurements see Blum, Kirby-Smith, and

[5] The term "development" is used herein as earlier (*4*) to include whatever changes take place which lead to the formation and growth of a tumor.



TEXT-FIGURE 1.—Distribution of the logarithm of the tumor development time t_d, in a population of genetically homogeneous (Strain A) male mice, exposed to ultraviolet radiation, the exposures being placed at regular intervals up to the time of tumor appearance. The data are from 12 experiments including a total of 676 mice. The dose and interval between doses was the same for a given experiment but varied between experiments. The mean values of t_d varied accordingly, and in the figure all the points have been corrected to a common, mean value for each experiment, *i. e.*, *log* t_d=0. The smallest number of animals in any single experiment was 41, the largest number 98; the solid disks represent the latter experiment.

The drawn curve is the integral of a normal distribution, with standard deviation 0.081 log days. The same distribution was found for a number of additional experiments in which smaller numbers of mice were used (*3–5*, *11*, *12*).

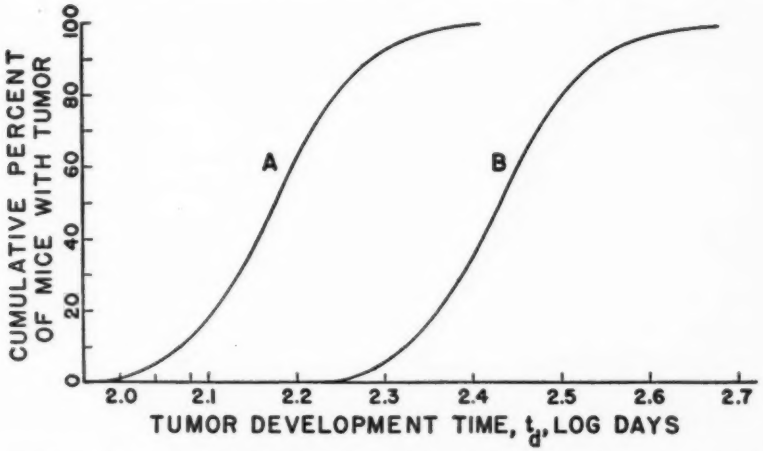
Grady (*14*)]. The number of these units per dose is indicated herein by the symbol D.

Experiments have shown that t_d is independent of the intensity of the incident radiation, at least down to a level of intensity considerably below those that were used in obtaining any of the data for the present analysis (*3*).[6]

II. 4. Summary of Data

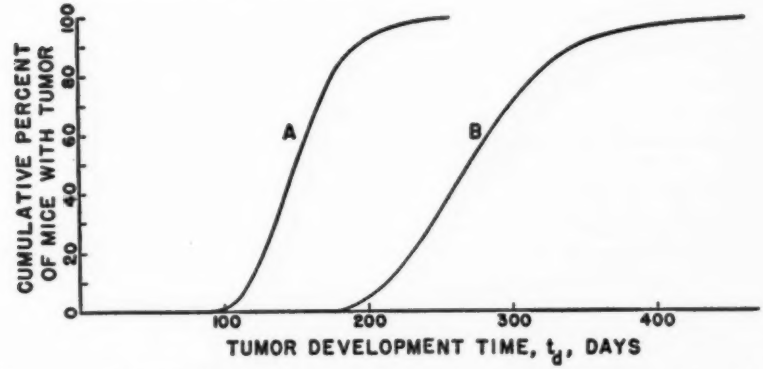
It may be convenient to summarize briefly the experimental data. The basic measurement is the "development time"—symbolized by t_d—which is measured as the time from the first dose of ultraviolet radiation to the appearance of a tumor of volume$\cong$100 mm^3. The quantity t_d varies with the dose D of radiation applied, and the frequency of applica-

[6] At very low intensities t_d appears to increase slightly as intensity increases, but this may result from a slight deviation from spectral neutrality of the filters employed to adjust the intensity of the polychromatic radiation (*10*, *15*). Whatever the nature of this apparent failure of reciprocity, it should not affect the present analysis.



TEXT-FIGURE 2.—Percent incidence curves for two experiments, A and B, plotted against *log* t_d. Curve B might represent a lower dose D or a longer interval between doses i than does curve A.

Note that the shape and slope are the same for both experiments, although the development time is longer for B than for A.



TEXT-FIGURE 3.—The same percent incidence curves represented in text-figure 2, but plotted against days instead of log days.

tion. The frequency is measured in terms of the interval i between any given dose and the next one. The analysis is based chiefly upon the way in which t_d varies as D or i is varied. The experiments may be divided into two groups:

a. Those experiments in which the mice received equal doses of radiation on a regular schedule maintained until tumors appeared, *i. e.*, throughout the whole of t_d. In this case the distribution of *log* t_d within a population of identically treated mice is normal, and has the same standard deviation for any value of D or i. Three schedules of exposure were used; 1, 5, and 7 days per week; the 5-per-week schedule being one dose each

day except Saturdays and Sundays. The data for the three schedules are given in table 1 and are plotted in text-figures 6 and 7, on the basis that t_d represents the time of 50 percent tumor incidence. Because of the logarithmic nature of the distribution (see text-figs. 1 and 2) the same relative relationships shown in text-figures 6 and 7 would be found if any other percentage incidence were chosen for comparison.

b. Those experiments in which a regular dosage schedule was maintained, but was discontinued before tumors appeared. If dosage was discontinued early no tumors ever appeared; if continued longer, tumors appeared but t_d increased inversely with the length of time the regular dosage schedule was maintained. If the schedule was interrupted for a time and then resumed, t_d was increased, the amount of increase depending upon the time at which the respite from dosage occurred. In all such cases the distribution of t_d is distorted and the variance is greater than when the dosage schedule was maintained throughout t_d. Data from such experiments are presented in table 5, and text-figures 8 and 9.

III. THEORETICAL

One property of cancer is rapid rate of growth. During at least a part of its development the cells of a tumor must proliferate at a faster rate than do the normal cells from which they originate. Growth rate may be treated quantitatively, and is the property which is considered in the present analysis.

III. A. RELATIVE GROWTH

Since tumors grow as a result of the proliferation of cells, the rate of tumor growth should always be some function of the number of existing, actively dividing cells. In general, the individual tumor cell does not enlarge beyond a certain quickly attained volume; so the rate of growth of a tumor is also a function of its volume. Hence, the growth of a tumor may be described by giving its volume V, or, alternatively, the number of cells composing it N, as a function f of the time t.

$$V=f(t);\ Nv=f(t) \tag{1}$$

where v is the average volume of the individual tumor cell, which is assumed to be constant. *Any exact understanding of the process of tumor development requires some knowledge of how this function depends upon the conditions under which the tumor grows.* The character of this function during the earlier stages of tumor development is indeterminable by present methods, which provide no means of detecting the existence of a tumor until it is composed of a relatively large number of cells—say, about 10^4—and hence, as pointed out earlier, any contemporary hypothesis regarding carcinogenesis must be, in a quantitative sense, an extrapolation.

For purely illustrative purposes we may consider the simplest type of growth; not because it fits the present data—we shall see shortly that it clearly does not—but because it provides a basis for illustration and for

subsequent reasoning. The simplest possible type of growth would be growth at a constant rate. Let us assume that the tumor grows at a *constant* relative rate, after somehow attaining a volume V_o which we may think of as the initial volume, *i. e.*, the volume when $t=o$. We may express this by

$$\frac{dV}{dt}=GV \tag{2}$$

where G is a constant, which may be called the growth constant, and integrating we obtain

$$ln\,\frac{V}{V_o}=Gt \tag{3}$$

in which ln is the natural logarithm, and V is the volume at the end of time t. Similarly, if it is assumed that a tumor is composed of a large number of cells, and that these are dividing at random with respect to each other but at a constant rate, growth is described by

$$\frac{dN}{dt}=G'N \tag{4}$$

and

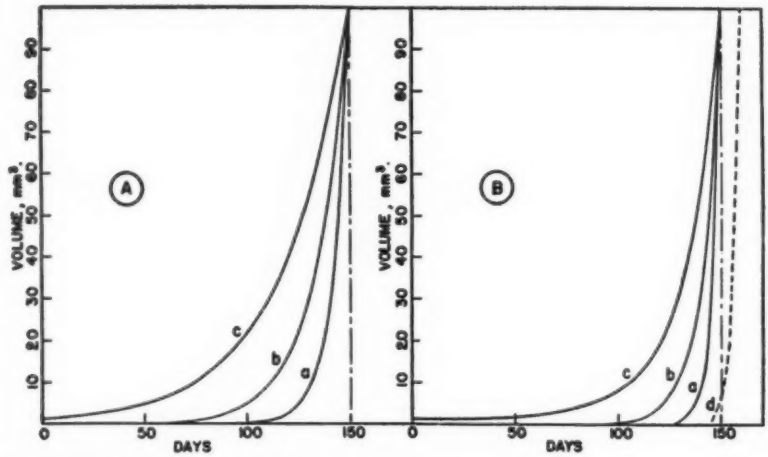
$$ln\,\frac{N}{N_o}=G't. \tag{5}$$

In the present case, G' may be treated as numerically equal to G, the same numerical values being arrived at whether one deals with volumes or with numbers of cells.

Let us examine the consequences of the assumed constancy of relative rate of tumor growth, in terms of the situation encountered in the present experiments, where the tumor has a volume V_d of approximately 100 mm^3 at the time t_d. For purposes of illustration, t_d will be taken as 150 days, a value which is consistent with our experiments. We may then assume any given volume, V_o, for the tumor at the time of applying the first dose of ultraviolet radiation, and calculate the shape of the growth curve throughout the course of the tumor's development. This has been done for three values of V_o in text-figure 4A, using equation (3). Curve a in this figure assumes that growth starts from a volume of 10^{-6} mm^3, or approximately the volume of one cell. Upon casual examination this curve suggests that there is a long period of time—about 100 days—during which nothing happens; but actually the volume at the end of 100 days, 0.2 mm^3, is too small to be detected grossly in the living animal or to be represented on the graph. Suppose, then, that the tumor originated as a single cell and grew at a constant rate. No tumor would be grossly observable until about 100 days, at the earliest, after the advent of the first tumor cell. It might be thought that the first 100 days represented an "induction" period during which, at some undefined point, the first tumor cells were formed; whereas the last 50 days represented a "growth" period. Is this not the kind of observation and reasoning upon which

numerous hypotheses involving "induction" and "growth" periods have been based? If so, it is obvious that these are subject to serious question. (See sec. III. C. 1. for further discussion of this point.)

To illustrate further, note curve b in text-figure 4A, where it is assumed that the tumor has a volume of 10^{-2} mm³ at the beginning of the curve, corresponding to approximately 10,000 tumor cells. In this case, the tumor growing at a constant rate throughout t_d would not reach an observable volume until after 50 days. In the case of curve c, a just observable



TEXT-FIGURE 4.—Hypothetical growth curves.

A. Growth following the simple equation of relative growth.

curve	initial volume, V_o
a	10^{-6} mm³
b	10^{-2} mm³
c	1 mm³

B. Progressively accelerated growth curve.

curve	initial volume, V_o
a	10^{-6} mm³
b	10^{-2} mm³
c	1 mm³
d	10^{-6} mm³ (rate slightly less than for a).

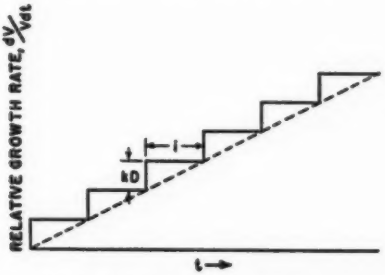
volume of 1 mm³ is assumed at the beginning of t_d. The rates of growth are, of course, quite different for the three curves, that for curve a (G= 0.127) is close to the average estimate for our experimental tumors when large enough to measure, *i. e.*, around the time the tumor reaches a volume of 100 mm³. (See sec. III. B. 3.)

A careful consideration of these relationships illustrates the necessity of taking into account the quantitative aspects of tumor growth, if one is to think clearly about the mechanism of carcinogenesis. It is not to be expected that the simple growth relationships described by equations (2) to (5) hold throughout the development of the tumor, and it may be easily shown that they do not in the case of the present experiments. This

discussion of the relative growth rate has been inserted to show the importance of understanding the quantitative growth relationships. It does not represent what happens in the case of tumors induced by ultraviolet radiation where, as will be seen, modification of the basic equation is required in order to fit the experimental data.

III. B. THEORY OF PROGRESSIVE ACCELERATION OF TUMOR GROWTH [7]

At this point the assumption will be made that the rate of tumor growth is progressively accelerated by successive doses of ultraviolet radiation. This is the basis of the tentative theory of carcinogenesis introduced in this paper. The success of the theory in describing the data will be discussed in the present section, as will its specific limitations. Whatever value the theory may have must depend upon its fitting the data better



TEXT-FIGURE 5.—Diagram to illustrate progressive acceleration of relative growth rate. (See text, sec. III. B. 1.)

than alternative ones, and the difficulties encountered by some of the latter will be the subject of a later section (III. C).

III. B. 1. BASIC MODEL

Let us now formulate an approximate model to describe progressive acceleration of growth rate. To begin, equation (2) may be rewritten in the form

$$\frac{dV}{Vdt}=G \tag{6}$$

The left-hand member of the equation now represents the *relative* instantaneous growth rate. If we think of the tumor as growing by the proliferation of tumor cells, this means that the proliferation of the cells occurs at a constant average rate. The relative instantaneous growth rate may be plotted against time as in text-figure 5; if this rate remained constant—that is, if equation (6) were obeyed throughout—the line representing the growth rate would be parallel to the abscissa.

Let it now be assumed that upon receiving each dose of ultraviolet radiation the rate of tumor growth is increased by an amount roughly

[7] Presented in preliminary form before the American Association for Cancer Research, April 1948 (5).

proportional to the dose, where the factor of proportionality may depend upon the past history of the course of exposures, but does not change rapidly from day to day. This may be illustrated as in text-figure 5 where the growth rate is indicated as rising abruptly to another level with each dose, the new rate being maintained until the next dose is received. Without assuming that this diagram pictures the exact happenings in a tumor, we may accept it as an approximation which is described by the following equations. If the increase in rate is directly proportional to the dose per exposure D, then during any given interval, i, between doses, the rate is constant and proportional to the number of doses, n, that have been given, so that if k is a proportionality factor

$$\frac{dV}{Vdi}=knD \tag{7}$$

and by integration

$$ln\frac{V_{n+1}}{V_n}=knDi \tag{8}$$

where V_n and V_{n+1} are the volumes at the beginning and end of this interval.

The number of doses is equal to the number of intervals, and when the dose and interval are constant the growth during n intervals is described by

$$ln\frac{V}{V_0}=kDi(1+2+3+\ldots n) \tag{9}$$

which on summing the arithmetical progression becomes

$$ln\frac{V}{V_0}=kDi\left(\frac{n^2+n}{2}\right). \tag{10}$$

For the time t covering n intervals

$$n=\frac{t}{i} \tag{11}$$

and substituting in (10) we obtain

$$ln\frac{V}{V_0}=kD\left(\frac{t^2}{2i}+\frac{t}{2}\right) \tag{12}$$

For high values of t, such as for t_d in our experiments, to a very close approximation

$$ln\frac{V}{V_0}\cong\frac{kDt^2}{2i} \tag{13}$$

The same approximation is arrived at by assuming a constant acceleration of growth rate as indicated by the dotted line in text-figure 5, which is described by

$$\frac{dV}{Vdt}=\frac{kDt}{i} \tag{14}$$

which, when D and i are constant, integrates to

$$ln\frac{V}{V_0}=\frac{kDt^2}{2i} \tag{15}$$ [8]

[8] Throughout this treatment the rate of growth is assumed to be negligible at the time of the first dose.

Equation (15) may be used to describe tumor growth when t has high values, but it does not hold exactly for short periods. Both methods of describing this relationship will be used in the following discussion, as convenience dictates.

III. B. 2. Development Time

The development time t_d (already defined in sec. II. 2) is taken as the time from the first exposure until the tumor has reached a given volume, herein designated V_d. Thus, V_d always has the same value, and for this specific case equation (15) may be written

$$ln\frac{V_d}{V_o}=\frac{kDt_d^2}{2i} \tag{16}$$

If it may be assumed that V_o is the same for all experiments (which is equivalent to saying that the tumor always starts from the same number of cells), $\frac{V_d}{V_o}$ is a constant at the time t_d. Hence, for any series of experiments in which i is maintained constant, t_d should vary inversely as the square root of the dose D, since by rearranging (16) we obtain

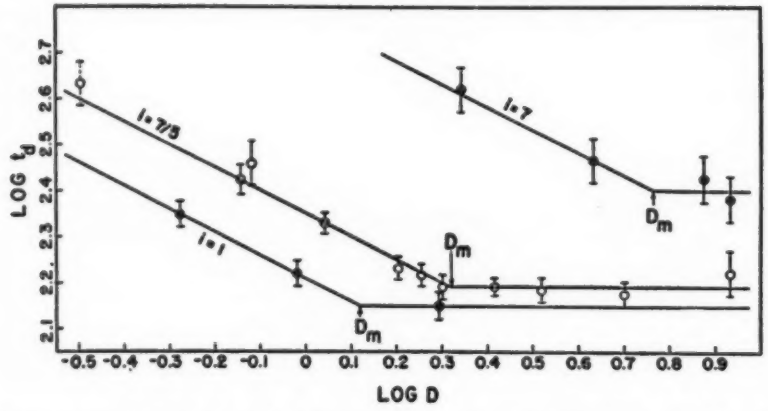
$$t_d=\left[\frac{2i}{k}ln\frac{V_d}{V_o}\right]^{\frac{1}{2}}D^{-\frac{1}{2}} \tag{17}$$

and all the values within the bracket are constants. Text-figure 6 shows the result of applying this relationship to the data. Here values of t_d are plotted against D, log-log coordinates being used. The values of t_d are based on the time to 50 percent incidence [9] of tumors in groups of identically treated mice. In 11 of the experiments the doses were applied 5 days per week, the interval being taken as 7/5 days and the curve so labeled in the figure. Above a certain value of D, designated D_m, t_d does not decrease with increase in D; the curve is a horizontal line. Below D_m the points are quite well fitted by the drawn curve which has the slope $-\frac{1}{2}$, representing the condition, explicit in equation (17), that t_d varies inversely as the square root of the dose. The reason for the break in the curve at D_m, will be taken up in section III. B. 4.

Experiments with dosage intervals of 1 and of 7 days are also represented in text-figure 6. The curves drawn resemble that for the 5-day-per-week schedule, that is, above a certain value, D_m, t_d remains constant as D increases, but for values below this the curve follows equation (17) in having the slope $-\frac{1}{2}$. There are few points involved in these latter curves, insufficient alone to establish the shape assigned to them, but their agreement with the curve of the 5-day-per-week schedule supports the analysis. The fit of the equation to the data is also shown in text-figure 7.

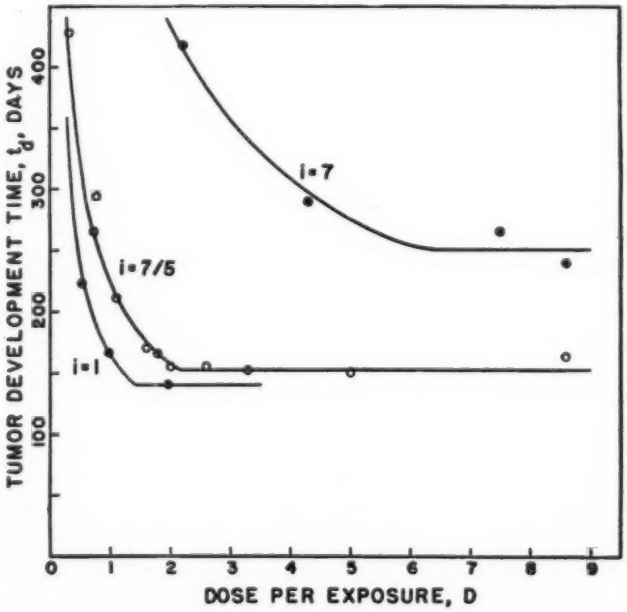
---

[9] Throughout the rest of the paper t_d will be the value for 50-percent incidence of tumors except where otherwise stated. Because of the nature of the incidence curves, the same relationship will hold for any other percent incidence. (See sec. II. 2.)



TEXT-FIGURE 6.—Relationship between dose D and development time t_d for three different intervals i.

The relative accuracy of the points is indicated by the vertical lines, which represent the limits which should be exceeded only once in 20 times on the basis of chance alone.



TEXT-FIGURE 7.—Relationship between dose D and the development time t_d for three different intervals i. The drawn curves follow the theory of progressive acceleration of tumor growth discussed in the text. The data and curves are the same as those plotted in a different manner in text-figure 6.

In table 1 the basic data are presented, and certain values calculated therefrom. Rearranging equation (16)

$$\frac{1}{k} ln \frac{V_d}{V_o} = \frac{Dt_d^4}{2i} \tag{18}$$

it is seen that $\frac{Dt_d^4}{2i}$ should be constant, since the left-hand member of the equation is composed of constants, and from column 6 in the table this is found to be true for doses below D_m (see text-fig. 6) for each given interval.

TABLE 1.—*Relationships between dose* (D), *interval* (i) *and development time* (t_d) (*data for 50-percent incidence of tumors*)

Experiment	Number of mice	D	i	t_d	$\frac{1}{k} ln \frac{V_d}{V_o}$	
					A. (equation 18)	B. (equation 19)[1]
BEF	47	1.97	1	141	19,583	19,444
BGH	48	.97	1	166	13,364	13,284
BIJ	41	.53	1	223	13,178 [2] Av. 13,278	13,119 [2] Av. 13,208
A	24	8.6	1.4	166	84,635	81,298
CMN	51	5.0	1.4	150	40,178	39,230
BFN	63	3.3	1.4	152	27,229	26,311
ARSTU	98	2.6	1.4	155	22,308	21,593
CGO	63	2.0	1.4	155	17,161	16,532
CHIOP	86	1.8	1.4	165	17,501	16,952
AAEI	70	1.6	1.4	170	16,514 [2] Av.	16,298 [2] Av.
ABFJ	67	1.1	1.4	212	17,656 17,647	17,275 17,167
H	22	.76	1.4	287	22,399	20,873
AGK	42	.72	1.4	265	18,058	17,898
AL [3]	22	.32	1.4	428	[3] 20,935	[3] 20,323
J	23	8.6	7	240	35,380	34,357
AN	20	7.5	7	266	40,575	36,907
AO	23	4.3	7	290	31,231 [2] Av.	25,211 [2] Av.
AP	20	2.2	7	418	27,456 29,475	28,182 26,362

[1] The data in this column are corrected for days lost, etc.
[2] Averages, weighted for number of animals, includes only values below D_m.
[3] t_d for this experiment was obtained by extrapolation from a small number of points, and hence is not included in the averages.

Rearranging equation (9)

$$\frac{1}{k} ln \frac{V}{V_o} = D(i+2i+3i+\cdots ni) \tag{19}$$

we see that $\frac{1}{k} ln \frac{V_d}{V_o}$ can be calculated in another way. This is convenient when intervals of different length are used; the corresponding values of i being substituted in the proper position in the series. Thus, for example, in the 5-day-per-week schedule the members of the series follow the order ($1\times1+2\times1+3\times1+4\times1+5\times3+$ . . . *etc.*). When this schedule of dosage is maintained for long periods, as in our experiments, the average interval for the 5-dose-per-week schedule is very nearly that for 5 equally

spaced intervals per week, *i. e.*, $i \cong 7/4$. In some of the experiments with this dosage schedule, a few exposures were left out because of failure of apparatus or other difficulties, and in some of the others a short, terminal period has been included in t_d; appropriate correction has been made in calculating the values which appear in column 7 of table 1 by means of equation (19). The values in column 7 are in good agreement with those in column 6, but may be considered to be slightly more exact, and are used in place of $\frac{Dt_d^3}{2i}$ hereafter.

It has been assumed in the above treatment that $ln \frac{V_d}{V_o}$ and k are both constants. Neither is directly determinable from the data, however, but the first may be assigned a reasonable value (see sec. III. B. 3), and a working value for the second may then be calculated. If this is done it is found that the experiments with different values of i, yield different values of k, the latter decreasing as the former increases.[10] This is not surprising. The model which was used in developing the equations assumes that k is independent of i; but this model is obviously oversimplified. There are numerous reasons for expecting k to be dependent upon the past history of tumor development, and i is a factor in that history. The representation of the relative rate of growth in text-figure 5 as rising abruptly at the time of the exposure and continuing unchanged until the advent of the next exposure is obviously diagrammatic and not likely to apply exactly to the actual process. If for some reason the rate of tumor growth fell off progressively during the interval, such an apparent discrepancy might occur. For example, destruction of tumor cells occurs when tumors regress, and although there is no direct evidence of regression in the present case it is probable that such destruction does go on. If destruction followed the course of proliferation instantaneously, and exactly, k need not vary with i, but it seems more likely that the destruction would be out of phase with proliferation so that the relative destruction per interval could vary with the interval, leading to a relationship such as that observed.

A factor which changes progressively with successive exposures to ultraviolet radiation, and which could affect the rate of tumor development, is the thickening of the epidermal tissues, and to a certain extent the deeper layers. Marked thickening of the corneum or horny layer of the epidermis reduces the amount of this radiation reaching the viable tissues beneath (*23*). While thickening of the corneum probably continues throughout the whole of the development time, it is more pronounced in early stages, and probably is nearly, if not completely, balanced by desquamation later on. Here, then, is a factor which should have a greater effect at some stages of tumor development than at others. If, as may be expected, the thickening of the corneum is proportional to the dosage,

[10] Empirically it is found that

$$\frac{Dt_d^3}{2i} = 2.555 \times 10^4 - \frac{1.554 \times 10^4}{i} \qquad (20)$$

but this relationship seems to have no theoretical implications.

it need not affect the general relationships observed, since measurement is always made at a given stage of tumor development. Proliferation of the epidermis does not begin immediately after exposure to ultraviolet radiation, however, there being no appreciable changes for about 24 hours. Maximum proliferation is not reached for a week or more and maximum thickening of the corneum somewhat later. Thus, doses of ultraviolet radiation falling within the first week, for example, would not be much restricted in penetration, due to the thickening of the corneum; whereas the penetration of doses applied later might be considerably affected. It would be difficult to predict the quantitative results when different schedules of dosage were used, but such an effect might account, in part at least, for the differences in the apparent value of k with different intervals.

Numerous other factors may contribute to the dependency of k on i, but it would be difficult to evaluate their relative importance. At any rate there seems no reason for abandoning, because of this deviation from the simple model relationship, the theory that tumor growth is progressively accelerated by repeated doses of radiation.

III. B. 3. Rate of Growth

At this point it may be asked whether the theory is compatible with the actual rate of growth of these tumors at the time when they are large enough to measure. An approximate answer may be given, since estimates of the growth rate of several hundred tumors are available (*6*). The growth rates were calculated for assumed constant relative rate, following equation (6). For the most trustworthy measurements the average relative rate $\left(\frac{dV}{Vdt}\right)$ was 0.119 with a standard deviation of 0.048; for the fastest growing tumor the value was 0.251.[11] The average value corresponds to a doubling of tumor volume in about 6 days, the maximum to doubling in less than 3 days.

According to our theory the instantaneous relative growth rate is given by equation (14). All the values for this equation are obtainable from the experiments except k, and this could be obtained from equation (16) if $\frac{V_d}{V_o}$ were known. Estimates of the latter can be made; for example, accepting the estimate of 10^8 cells for V_d (the approximate number in a tumor of 100 mm^3 volume), and assuming that the tumor started from a single cell, $\frac{V_d}{V_o}=10^8$. In table 2 are presented values calculated for the expected growth rate for representative experiments, when reasonable values are assumed for $\frac{V_d}{V_o}$. It is noted that the calculated growth rates $\left(\frac{dV}{Vdt}\right)$ are in fair agreement with those actually measured (average

[11] The values originally published are based on common logarithms and should be multiplied by 2.303 to obtain those presented herein, which are based on natural logarithms.

value=0.119) and, also, that the value of $\frac{V_d}{V_o}$ is not highly critical. In general, the rates calculated on the basis of our theory are higher than those based on actual measurements. Measurements made much before the tumor reached a volume of 100 mm³ were unreliable because of the small dimensions, and by the time a tumor had grown beyond this volume its growth rate tended to diminish. Thus, in most cases the estimates based on these measurements are probably lower than the actual rates at time t_d or just before. The use of equations (3) and (6) for estimating the rate instead of the equation for accelerated growth would yield lower values than predicted by the theory, but the uncertainty of the measurements does not justify a more rigorous treatment. Another factor which might have tended toward a low average value is that a good many of the tumors, when measured, had not been exposed to ultraviolet radiation for some time; and on the basis of our theory the rate of growth in such cases should be less than when the exposures were continued up to the time t_d (see sec. III. B. 5.). The above factors should be taken into account in making the comparison between the rates estimated from measurements of tumor volume and those calculated on the basis of our theory.

TABLE 2.—*Calculated tumor growth rates at time* t_d *(50-percent tumor incidence) for several experiments*

Experiment	D	i	t_d	$\frac{V_d}{V_o}$	Instantaneous relative growth rate $\left(\frac{dV}{Vdt}\right)$ at t_d
BGH	0. 97	1	166	10^7 10^8 10^9	0. 19 . 22 . 26
BIJ	0. 53	1	223	10^7 10^8 10^9	0. 14 . 17 . 19
CGO	2. 0	1. 4	155	10^7 10^8 10^9	0. 20 . 23 . 26
AKG	0. 72	1. 4	265	10^7 10^8 10^9	0. 13 . 14 . 16
AO	4. 3	7	290	10^7 10^8 10^9	0. 10 . 12 . 14
AP	2. 2	7	418	10^7 10^8 10^9	0. 08 . 09 . 10

Calculated growth rates for a single experiment for various percent incidences are presented in table 3; the estimated growth rates are not very much higher for the early than for the late appearing tumors. At the time the measurements on growth rate were reported (*6*) it was an-

ticipated that appropriate grouping according to experimental conditions would reveal differences in growth rates, *e. g.*, for a given dosage schedule faster rates were expected for earlier appearing tumors, but no theory was available at the time for predicting the amount of difference. No such differences were observed (*6*) but when one considers the relatively narrow range of the rates indicated in tables 2 and 3, and the wide variations in the measurements, this is perhaps not surprising. Without a more adequate means of measuring of growth rate than is available, it seems unlikely that significant differences would be observed.

Considering all the factors which tend toward low measured values for growth rates, the uncertainty of those measurements, and of the value $\frac{V_d}{V_o}$, the agreement between the observed and calculated data may be quite good. Certainly the discrepancies are not great enough to threaten the validity of the theory, particularly when, as indicated in the preceding section, exact adherence to the predicted growth rate throughout the development time is not essential nor to be expected. (See sec. III. B. 2.)

TABLE 3.—*Calculated tumor growth rates for experiment* C60 *at time* t_d *for various percentage incidences;* $\frac{V_d}{V_o}$ *taken as* 10^8

Percentage incidence	t_d	Instantaneous relative growth rate $\left(\frac{dV}{Vdt}\right)$, at t_d
10	121	0. 30
20	132	. 27
30	141	. 26
40	148	. 24
50	155	. 23
60	163	. 22
70	171	. 21
80	181	. 20
90	199	. 18

Let us now examine some aspects of progressively accelerated growth rate by means of graphic representation. Curve a in text-figure 4B is an accelerated growth curve following equations (14) and (15) in which $\frac{V_d}{V_o}$ is taken as 10^8 and t_d as 150 days. The tumor would first be grossly detectable about 20 days before it reached the volume 100 mm^3, agreeing with the observation that on the average these tumors are not grossly manifest until 2 or 3 weeks before they reach this volume. The small volume so late in development of the tumor helps to explain the fact that the exposures to ultraviolet radiation can continue to accelerate tumor development almost up to the end of t_d (see sec. III. B. 5.), although the radiation cannot penetrate effectively after the tumor reaches a grossly detectable volume.

To illustrate another aspect, let us assume that every tumor develops from a single cell whose growth rate is accelerated by ultraviolet radiation, and that the growth rate of the "clone" resulting from this cell is pro-

922686—51——2

gressively accelerated by successive doses of radiation. Let us imagine that there are two clones, one growing thus at a "fast," the other at a "slow" rate. The growth rate of the fast clone need exceed that of the slow clone by only a very little in order to outstrip it; and thus the ultimate tumor might arise almost exclusively from the fast clone, although the slow clone was undergoing the same process of acceleration. Referring again to text-figure 4B, the broken curve d is based on the same value of $\frac{V_d}{V_o}$ as is curve a, but is calculated for a slightly lower acceleration. In terms of equation (14) the values of $\frac{kD}{i}$ are 1.6×10^{-3} and 1.4×10^{-3} for curves a and d, respectively. Curve d arrives at volume 100 mm^3 only 10 days after curve a; but at 150 days when curve a reaches this value, curve d is only about 10 percent as high. Thus, the slow clone represented by curve d would only contribute 10 percent to the total tumor volume at time t_d. If it is assumed that there is some sort of distribution of the rates of growth of the tumor cells, only those growing near the maximum rate would contribute appreciably to the tumor volume.

Although the picture of separately accelerated clones serves to illustrate an important quantitative relationship, it is unlikely that it approaches very closely to reality. A basically different picture which fits the quantitative measurements as well, but which may approach the true mechanism more closely, is therefore suggested. Let us suppose that the cells composing the tissue which is about to receive a first dose of radiation are proliferating at a slow rate characteristic of normal tissue. The first exposure results in an increase in the rate of proliferation of all the cells of the tissue. At the same time there is a tendency for the cells to return to their normal rate of cell division, presumably due to factors controlling the rate of proliferation, such as are generally postulated to exist in any organized tissue. There would, as successive doses were applied, be a tendency toward acceleration, resulting from the action of the radiation. This would be opposed by a tendency toward return to the normal rate, resulting from the normal controlling action of the tissues. A certain balance should result in the greater part of the tissue, but in a few foci acceleration might exceed deceleration. These foci would be potential tumors. As in the case of the clones used above for illustration, the rate of growth within a given one of these foci would only have to exceed by a very little that of another focus in order to outstrip it in growth.

If there were several foci of tumor cells in close proximity, the one which proliferated most rapidly might outstrip the others, so that the tumor ultimately formed would be composed almost entirely of cells arising from this focus. The same argument would apply, although less radically, if relative growth rate were constant rather than accelerated (*e. g.*, refer to text-fig. 4A). Thus, the question whether tumors originate from single or multiple foci—one which often arises in discussions of carcinogenesis—would be difficult to answer experimentally, and perhaps has little meaning.

Up to this point the proliferation of "tumor" cells has been spoken of without reference to what distinguishes these cells from the "normal" tissue cells. It is commonly assumed that a carcinogenic agent produces a new type of cell, *e. g.*, a mutant, which is sharply differentiable from the normal cell type by certain characteristics. Following this reasoning one should be able to say that a given cell is a tumor cell, another not, and could presumably assign a time of origin, *i. e.*, the time at which the transition is made from a normal to a tumor cell. Up to the present, such a differentiation has not been made experimentally. It seems reasonable, therefore, to suggest that tumors are composed of essentially normal cells which differ from their fellows in that they proliferate at a faster rate, and in other associated characteristics which determine, for example, the "invasiveness" of malignant tumor tissue. Let us see how this concept fits with the theory under discussion.

A single dose with ultraviolet radiation results in a general increase in the rate of cell proliferation in mouse skin, as indicated by thickening of various layers. If there is no further interference, the hyperplasia diminishes and the tissues tend to return to their normal dimensions. With successive dosage, however, at least some degree of hyperplasia is maintained (*4*). Let us suppose that successive doses of ultraviolet radiation tend to accelerate proliferation progressively in all the tissue cells, but that the susceptibility to acceleration is not equally distributed. Some foci of cells may be, for some reason, unusually susceptible to this acceleration, or have greater ability to escape from the controlling factors of normal tissues. Such a focus may, then, outgrow the others and become a tumor, for, as has been pointed out above, a small difference in rate may permit one focus rapidly to outgrow another. The more rapidly proliferating cells making up the tumor would then be recognized as "tumor" cells although differing only in degree from the normal ones.

III. B. 4. Maximum Limit of Growth Rate

It is obvious that there must be a maximum rate of growth which cannot be exceeded, since physiological factors, for example, the rate at which materials for growth can be supplied, must set an upper limit. Hence, progressive acceleration of growth, which is the essence of our theory, could be pushed only to a certain point; and, acceleration being directly proportional to dose, a maximum should be reached beyond which increase in dose would not increase the rate of development. This appears to be what happens, since each of the curves in text-figures 6 and 7 becomes horizontal at a certain value of D which has been designated D_m.

If the limiting factor is maximum rate of growth, and is set by factors independent of the radiation itself, $\frac{dV}{Vdt}$ should be the same at all three values of D_m. This possibility is tested in table 4. From equation (14) it is seen that if the over-all growth rate were the same at all three values of D_m, the same value of $\frac{kD_m t_d}{i}$ should obtain, but this is not the case.

The quantity $\frac{D_m t_d}{i}$ is more nearly a constant, suggesting that the maximum rate of growth is more closely related to the number and magnitude of the accelerations. It seems probable that the minimum values of t_d in the three curves represent a common maximum rate of proliferation which cannot, for physiological reasons, be exceeded. The limiting factor would seem to operate throughout the whole of time t_d. Else, the curve would not be horizontal beyond D_m, and t_d would continue to fall off as D increased; for, lacking such continuous limitation, the maximum rate of growth would be reached earlier at higher doses. This could be explained by assuming, for example, that the limiting factor is the concentration of building materials in the bloodstream, and that the effect of each dose of ultraviolet radiation is to increase the ability of the cells to utilize this material for growth.

The values of D_m for table 4 were taken from the curves in text-figure 6, which appear to break sharply. It seems unlikely that the breaks are as abrupt as this, and text-figure 7 suggests a more gradual transition. Thus the values used in the table may not be very exact. The range of uncertainty is, nevertheless, quite restricted, as may be seen by examining the curves and table 1.

TABLE 4.—*Relationships at* D_m *(Data for 50-percent incidence of tumors)*

i	t_d	D_m	[1] $\frac{kD_m t_d}{i}$	$\frac{D_m t_d}{i}$
1.0	141	1.44	0.283	203
1.4	155	2.04	.242	226
7.0	256	5.57	.143	204

[1] The value of k based on $\frac{V_d}{V_o}=10^3$.

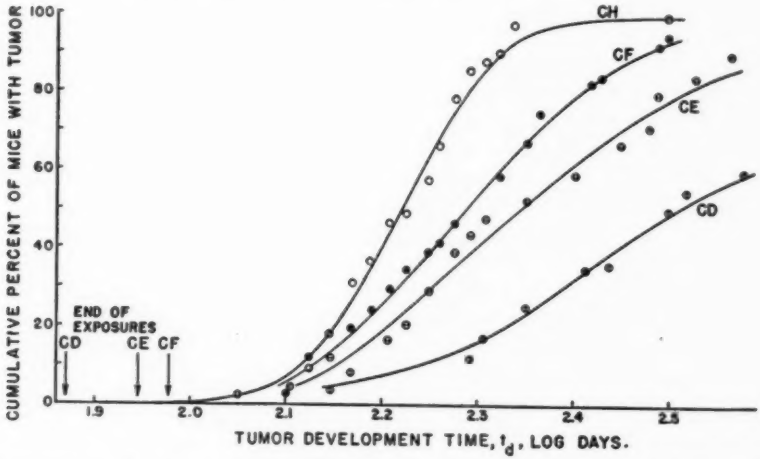
III. B. 5. Effect of Interruption of Dosage Schedule

According to the above analysis, if the exposures were discontinued a tumor should continue to grow at the relative rate which was established at the time the exposures ceased t_1. This may be expressed by

$$ln \frac{V_d}{V_0}=kD\ [(i+2i+3i+\cdots n_1 i)+(n_1+1)t_2] \qquad (21)$$

where n_1 is the number of exposures corresponding to t_1, and t_2 is the time during which growth continues at constant rate.

Such an experiment is described in text-figure 8, and the results of application of equation (21) to the data for 50-percent tumor incidence are presented in table 5 (exp. I). The observed values of t_d are greater than those calculated by means of equation (21), indicating that the rate of tumor growth is slower at some point than is predicted by the equation. Moreover, the discrepancy between t_d (obs.) and t_d (calc.) increases systematically with increase in t_d. Experiment II of table 5 was carried



TEXT-FIGURE 8.—Effect on development time of discontinuation of dosage. In series CF, CE, and CD the exposures were discontinued at the times indicated. In CH, the control, the dosage continued until the appearance of tumors.

TABLE 5.—*Effect of interrupting schedule of dosage*

[For all experiments, data are for 50 percent tumor incidence]

[Experiment I. D=1.8; i=1.4. This is the experiment described in text-figure 8. All the series were run simultaneously]

Series	t_1	t_2*	t_d(obs.)	t_d (calc.)
CH	166	----------	166	164
CF	95	100	195	185
CE	88	126	214	192
CD	74	238	312	212

[Experiment II. D=5.0 (but calculated as D_m=2.05); i=1.4. Both series were run simultaneously]

Series	t_1	t_2*	t_d (obs.)	t_d (calc.)
CM	149	----------	150	155
CL	89	93	182	179

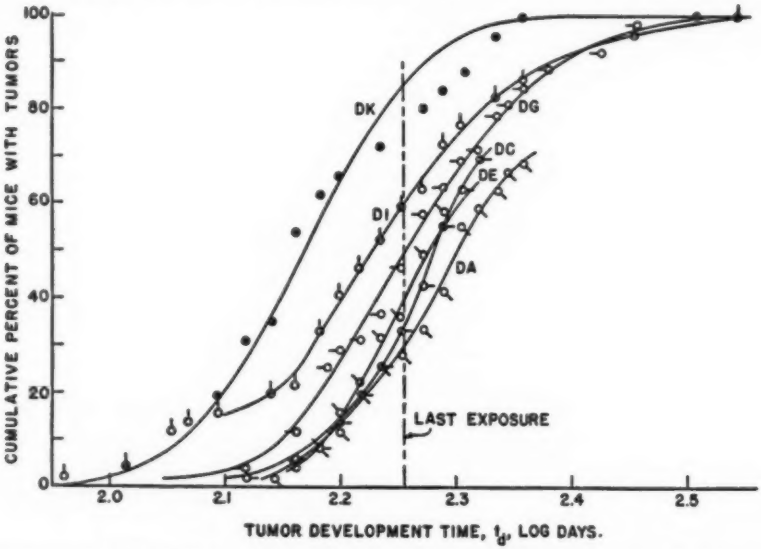
[Experiment III. D=2.0; i=1.4. All series were run simultaneously]

Series	t_1	t_2*	t_3	t_4*	t_d (obs.)	t_d (calc.)
DK	155	----------	----------	----------	155	155
DI	75	30	64	----------	169	170
DG	47	30	103	----------	180	173
DE	33	30	117	9	189	176
DC	19	30	131	11	191	178
DA	5	30	145	20	200	181

*Periods without exposures.

out in the same way as experiment I, the only important difference being that in the former D has a value well above D_m. Assuming, in accord with the discussion in section III. B. 4. that the acceleration of growth is the same for D_m and all higher doses, the value of D_m (2.05) has been used in the calculations indicated in table 5. The apparent agreement with experiment I obtained in this way offers some support to the concept of a maximum rate of growth.

In experiment III of the table, also described in text-figure 9, an initial period of exposures t_1 was followed by a rest period of 30 days t_2 after which



TEXT-FIGURE 9.—Effect on development time, of the interruption of dosage. In experiments DA to DI the exposures were discontinued for 30 days and then resumed. The arrangement of the periods of respite are indicated in table 6. The control DK, consisted of a smaller number of animals (26)—the curve drawn is based on pooled data from table 1.

the exposures were resumed for another period t_3. At the start of this experiment it was planned to prolong the exposure period t_3 until all mice developed tumors, but it became necessary to terminate the exposures at a certain date, so that in some of the series there is a terminal rest period t_4. According to the theory the following equation should apply

$$ln \frac{V_d}{V_0} = kD\{(i+2i+3i+\cdots n_1 i)+(n_1+1)t_2+ [(n_1+2)i+(n_1+3)i+(n_1+4)i+\cdots(n_1+n_3+1)i]+ (n_1+n_3+2)t_4\} \quad (22)$$

where n_1 is the number of exposures during t_1, and n_3 the number during t_3. As in experiment I when rest periods are introduced, the t_d observed

is generally less than that calculated. The difficulty of interpreting this experiment is not decreased by referring to the graphical representation in text-figure 9. For example, the curve for series DI suggests that the first 10 or 15 percent of mice to display tumors, develop them just as rapidly as if no 30-day rest period had been introduced, whereas later the incidence curve indicates a distinct delay, as is true for all incidences for the other curves; this could, however, be a chance variation.[12]

According to equations (21) and (22), t_d should be longer when rest periods are introduced, no matter at what point in development, and in experiments such as III, the earlier the rest period is introduced the greater should be the delay. Referring to the figures, it is seen that this is, in general, true. As a rule the rest periods seem to have more delaying effect than predicted, and the discrepancy is greater the earlier the rest period is introduced. Such discrepancies seem, in general, to be in the same direction as those described in section III. B. 2; and the same discussion may apply.

The experiments described in table 5 and text-figures 8 and 9 illustrate a point of considerable importance (*e. g.*, see sec. III. C. 2.); that the rate of development is still influenced by the exposures to ultraviolet radiation up to (or at least very near to) the time the tumors appear. For example, in series CD of experiment I, in which exposures ceased after 74 days, the appearance of the tumors was greatly delayed, and even stopping the exposures as late as 95 days (CF) resulted in a marked increase in development time over that of the control (CH) in which the exposures continued until the tumors appeared. In text-figure 9 will be noted the greater steepness of the curves when dosage is interrupted for a period, but subsequently continued, as compared to those in text-figure 8 where dosage once stopped is not resumed. This indicates the effect of regular exposures carried on in the later stages of t_d. Again, the fact that grossly detectable tumors could not be induced with a single exposure to ultraviolet radiation or even exposures covering a period of 2 weeks (*4*, *5*) shows the importance of repeated exposures. All these things are in qualitative agreement with the present theory but are incompatible with certain of those discussed below. Somewhat different qualitative relationships are reported by Rusch and Kline (*26*, *27*) for similar experiments, but their data are not described in a way which makes quantitative comparison with ours possible.

III. B. 6. Summing Up

Before considering other hypotheses regarding carcinogenesis, a summary of the present theory and a consideration of its strengths and weaknesses are in order.

To summarize briefly: Ultraviolet radiation causes acceleration of the rate of proliferation of the "tumor" cells, each dose of the radiation causing an increase in the relative rate of proliferation proportional to the

[12] Statistically, estimated at 50-percent incidence, all the series with rest periods (DI to DA) are significantly different from the control, at the P=0.05 level, and the difference between series DI and DA is also significant, but the distortion of the curves makes estimates at any particular incidence level somewhat uncertain.

magnitude of the dose, up to a certain limit set by physiological factors limiting the absolute growth rate. The "tumor" cells may not differ sharply from normal tissue cells, but represent those cells and their offspring whose growth rate is most effectively accelerated by the ultraviolet radiation, and which, hence, outstrip their fellows and form a tumor.

The principal strength of the theory is in the fitting of the data for given experimental conditions, by a simple equation based on the postulate that tumor growth is progressively accelerated by regularly repeated doses of ultraviolet radiation, the amount of acceleration being directly proportional to the dose.

For different intervals between doses, different constants must be used in this equation, the variation being systematic. Again, the simple equation does not fit exactly when the schedule of doses is interrupted. Neither of these discrepancies substantially weakens the basic theory, since the equation is an approximate one at best, but they indicate the existence of secondary processes such as were discussed in section III. B. 2. Lacking a quantitative explanation of these secondary processes, the theory remains, however, incomplete. Reasonable mechanisms may be presented to account for the observed discrepancies, but these have not been formulated in exact quantitative terms.

The theory accounts for the sudden appearance of tumors after a period of apparent "latency." The idea that the tumor cells are essentially normal cells differing only in a quantitative sense, is consistent with the cytological similarity between tumor and normal cells.

The theory is formulated in over-all quantitative terms and provides no specific biological mechanism, which may be regarded either as weakness or strength. The idea of accelerated growth does not, however, seem out of harmony with some of the happenings in embryological development where cell proliferation speeds up locally for limited periods. One could no doubt invent a considerable number of mechanisms, and for this reason none will be discussed, since there would be little chance at present of establishing its uniqueness.

At this point certain factors which might affect the quantitative relationships, should be mentioned. High doses of ultraviolet radiation cause lowered food intake and slower growth in mice (*13*), and such conditions are generally associated with increased tumor development time. However, the effect is not great at dosage levels below D_m. Tumor-development time increases somewhat with age of the mice, which again might affect the quantitative relationships. The recent finding that "visible" light (wavelengths 0.3 μ to 0.5 μ) promotes recovery from the effects of ultraviolet radiation, could affect our data. This possibility is particularly suggested by recent demonstration in this laboratory of such an effect in a vertebrate form (*Amblystoma*), although earlier studies failed to show it in human skin (*16*). There was no strict control of the room light in our experiments with mice, but there was little sunlight in the rooms, and the tungsten filament lamps used for illumination were deficient in the effective wavelengths. Moreover, it is the habit of mice to shun the light.

The reproducibility of our experiments at different times of the year (*5*), speaks against this factor as having significant influence. All in all it seems reasonable to assume all these factors to be trivial in their influence on the quantitative relationships, and this attitude has been adopted in the present analysis.

III. C. ALTERNATIVE THEORIES

Whatever its inherent strengths and weaknesses, a theory such as the foregoing must derive much of its support from the failure of other theories to fit the experimental data in a quantitative manner. For this reason, the attempt will now be made to formulate quantitatively some of the current ideas about carcinogenesis, to see how they fit these data. A review of the separate hypotheses will not be attempted.

III. C. 1. Separable Induction and Growth

No matter what the carcinogenic agent employed, tumors never appear until long after its first application. It is common parlance to refer to the lapse of time between application of a carcinogenic agent and the appearance of a tumor, as the "latent" or "induction" period. Either term implies that the parent tumor cells have not during this period assumed the characteristics which they will later display, and this implication is tacit in a good many of the hypotheses which have been formulated regarding carcinogenesis. It has already been pointed out, however, that there is no basis for assuming that tumor growth is not going on throughout the period that is generally characterized as "latent." Reference to text-figure 4A and the discussion thereof (sec. III. A) should make this clear.

Inherent in the present data are further objections to the concept of separable growth and induction periods. Hypotheses based on this concept usually contain the assumption—sometimes tacit, without direct statement—that the tumor cells proliferate in a more or less unrestricted fashion once they are formed. The tumor is often said to grow "autonomously." In such case, one would assume that the rate of proliferation of the tumor cells is inherent in these cells themselves; and if this were so there would be no relationship between their proliferation rate and the time required for their induction. However, as pointed out earlier (*4*), these data contain evidence that if there are separate periods of induction and growth they must be proportional to each other.

Let us assume, for purposes of argument, that the development time may be separated into distinct periods of induction and growth, and state this hypothesis symbolically as follows,

$$t_d = t_i + t_g \tag{23}$$

where t_i represents an "induction period" during which the tumor cells are formed, and t_g represents a "growth period" during which these tumor cells proliferate.

Borrowing symbols from equations (4) and (5) we may write,

$$t_g = f\,(N, G', \ldots) \tag{24}$$

to indicate that the growth period is a function of the number of cells, N, and of the rate of their proliferation, which is a function of G'. Equation (24) is indicated to be incomplete, because other unspecified factors might enter; thus we are not restricted to acceptance of the relative growth equation in its simple form.

Combining (23) and (24) we obtain

$$t_d = t_i + f\,(N, G', \ldots). \tag{25}$$

We are met with a serious obstacle in attempting to apply such an equation to the data. Examination of text-figures 1 and 2 shows that the tumor-incidence curves for a constant schedule of dosage always have the same shape and slope when plotted against the *logarithm* of t_d. Hence, it must be assumed that the shape and slope of the curves are determined by some common, constant relationship. To rationalize such a relationship with equation (23), the induction period t_i and the growth period t_g must always be proportional. It follows that the induction period would have to be a function of both the number of tumor cells and the growth rate, which are going to obtain during the subsequent growth period, *i. e.*,

$$t_i \propto f\,(N, G', \ldots). \tag{26}$$

Hence, before accepting the hypothesis that was assumed in setting up equation (23), one needs to invent some mechanism by which the induction period always adjusts itself so as to be proportional to the growth period that is going to follow it! Such a mechanism could hardly be consistent with the idea that the tumor cells proliferate autonomously at their own *inherent* rate once they are formed, for that rate must be determined by the same factors that determine the length of the induction period.

III. C. 2. Somatic Mutations

The somatic mutation theory of cancer, orginally formulated by Boveri in the early part of the century, has numerous attractive aspects, and has increased in favor during recent years. It postulates that tumor cells originate from normal tissue cells by mutation, thus assuming characteristics which distinguish tumor cells from normal cells. This concept is of particular interest as regards the experiments under consideration, since ultraviolet radiation of the same wavelengths that induce cancer is effective in inducing mutation in a multiplicity of living organisms.[13] The theory implies a virtually irreversible change from a normal tissue cell to a tumor cell which takes on new characteristics that it maintains thenceforward and transmits to its daughter cells. One of the outstanding characteristics of tumor cells is that in some stages at least they proliferate more rapidly than their normal fellows, and, since this aspect may be treated quantitatively, attention will be focused on it to the

[13] Indeed the present experiments have been cited by at least one writer [Rhoads (*35*)], as evidence for the somatic mutation theory but we see herein serious difficulties in accepting this point of view.

exclusion of others. The same quantitative relationships might be expected for any other irreversible, transmitted change. Let us examine these relationships in terms of the present data.

One of the facts clearly shown by experiment is that repeated doses of ultraviolet radiation are required to cause a tumor to appear (sec. III. B. 5). This might be interpreted to mean that a given cell must be repeatedly acted upon by the radiation before it mutates, but this seems improbable because typical mutations—induced by ultraviolet radiation or any other agent—occur suddenly. More important, perhaps, it has been shown above that the concept of an induction period followed by a period of unrestricted growth is incompatible with the data. Even if it is assumed that successive exposures are required to induce a mutation, the theory still meets with difficulties.

Since elsewhere among living systems a single dose of ultraviolet radiation produces a certain proportion of mutations, one may imagine that with each dose of such radiation applied to a mouse's skin a corresponding number of normal cells mutate to tumor cells. If it is assumed that the number of tumor cells so produced is small and their inherent rate of proliferation low, it is conceivable that no tumor would be detected within the lifetime of the mouse as the result of a single or a small number of doses of ultraviolet radiation. On the other hand, by repeating the dose many times at appropriate intervals, it is conceivable that enough tumor cells might be produced by mutation and by the proliferation of the mutants, to result in an observable tumor. Such a concept would fit *qualitatively* with the experimental finding that a single dose of ultraviolet radiation does not produce a tumor, but repeated dosage does. When the concept is examined *quantitatively* the conclusion is, however, quite the contrary.[14]

If N_a represents the number of tumor cells formed by the initial dose of ultraviolet radiation we see from equation (5) that

$$ln\frac{N_s}{N_a}=G't \tag{27}$$

where N_s is the number of these cells resulting from the initial dose at the end of time, t, that is, it includes the original tumor cells (N_a in number) plus all those formed subsequently in time, t, by proliferation. If a series of equal doses were given at regular intervals, as in our experiments, the time t might be considered to be divided into n intervals each preceded by a dose of ultraviolet radiation. Since i represents the interval between doses,

$$t=ni \tag{28}$$

Substituting from equation (28) in (27) and rewriting in exponential form,

$$N_s=N_ae^{G'ni}. \tag{29}$$

[14] This idea was developed in an earlier paper (4) the treatment used therein was in error, however, although the same general conclusion was reached.

Under the conditions outlined, each dose of ultraviolet radiation would result in the formation of N_a cells, and the total number of cells (N_s) would depend upon the length of time allowed for proliferation. The mutant cells (N_a) produced by the last dose of the series would only be able to proliferate during a single interval, i, and, since $n=1$, would contribute $N_a e^{G'i}$ cells to the total; the next to the last dose would contribute $N_a e^{G'2i}$ cells, since $n=2$, *etc.*[15] Thus we may write for the total cells N at the end of time t, including both those formed directly by the action of the ultraviolet radiation and their offspring

$$N=N_a(e^{G'i}+e^{G'2i}+e^{G'3i}+ \ldots e^{G'ni}). \tag{30}$$

This is a geometrical progression in which the first term and the ratio of increase are both equal to $e^{G'i}$. It can be summed to obtain

$$\frac{N}{N_a}=\frac{e^{G'i}(e^{G'ni}-1)}{e^{G'i}-1}. \tag{31}$$

Substituting t for ni from equation (28) in (31)

$$\frac{N}{N_a}=\frac{e^{G't}(e^{G't}-1)}{e^{G'i}-1} \tag{32}$$

In the present experiments a given number of cells N_d (corresponding to volume V_d) is reached in time t_d, and since t_d is always large, to a very close approximation,

$$\frac{N_d}{N_a}\cong e^{G't_d}\left(\frac{e^{G'i}}{e^{G'i}-1}\right). \tag{33}$$

Since reciprocity may be assumed (see sec. II. 3), the number of tumor cells formed per dose should be proportional to the dose, at least to a close approximation

$$N_a\cong AD \tag{34}$$

Where A is a proportionality constant, and substituting from (34) in (33)

$$\frac{N_d}{A}\cong De^{G't_d}\left(\frac{e^{G'i}}{e^{G'i}-1}\right). \tag{35}$$

Both N_d and A are constant, and hence substitution of the appropriate experimental values into the right-hand side of the equation should yield a constant value, if the hypothesis of somatic mutations holds. Examination of table 6 shows this to be far from the case, however.

To approach the problem in another way, let us assume a dose of ultraviolet radiation to be given each day ($i=1$), and that each dose induces one tissue cell to mutate to a tumor cell ($N_a=1$). Let each tumor cell divide every sixth day ($G'\sim 0.119$=the average measured growth rate

[15] Actually the intervals in some of the experiments are shorter than a single cycle of cell division, but this does not vitiate the general analysis.

for these tumors which no doubt errs on the side of conservatism in the present case). It is then a matter of simple arithmetic to calculate the total number of tumor cells at any time after the first dose. This has been done in table 7. It is seen that within a relatively short time after the first dose, the contribution of tumor cells by mutation becomes negligible compared to the number contributed by proliferation of those tumor cells already present. Substituting other reasonable values for the ones used in the example the result would be essentially the same. If tumor development were based on the accumulation of tumor cells by mutation and proliferation, doses of radiation would not affect tumor development as late as they are found to do.

TABLE 6.—*Application of the theory of somatic mutations to the experimental data (see text)*

Experiment	i	D	t_d	$De^{G'i}t_d\left(\frac{e^{G'i}}{e^{G'i}-1}\right)^*$
BEF	1	1.97	141	3.6×10^{8}
BGH	1	.97	166	3.4×10^{9}
BIJ	1	.53	223	1.6×10^{13}
A	1.4	8.6	166	2.1×10^{10}
CMN	1.4	5.0	150	1.8×10^{8}
BFN	1.4	3.3	152	1.5×10^{9}
ARSTU	1.4	2.6	155	1.7×10^{9}
CGO	1.4	2.0	155	1.3×10^{9}
CHIOP	1.4	1.8	165	3.6×10^{9}
AAEI	1.4	1.6	170	6.1×10^{9}
ABFJ	1.4	1.1	212	6.3×10^{11}
H	1.4	.76	287	3.3×10^{13}
AGK	1.4	.72	265	2.3×10^{14}
AL	1.4	.32	428	2.7×10^{23}
J	7	8.6	240	3.8×10^{13}
AN	7	7.5	266	7.3×10^{14}
AO	7	4.3	290	7.2×10^{18}
AP	7	2.2	418	1.5×10^{22}

*The value used for G' is the average value of G (=0.119), obtained from direct measurement of tumor growth. (See sec. III. B. 3.)

TABLE 7.—*Growth of a tumor; assuming that 1 tumor cell originates each day by mutation, and that all tumor cells divide every sixth day*

Time after first mutation (t)	Total number of tumor cells (N)	Number of cells from mutation / Number of cells from proliferation
1	1	1.0
6	7	.5
12	21	.33
18	46	.20
24	75	.11
30	132	.06
36	250	.03
42	480	.015
48	934	.0075
54	1,844	.0032

One can only conclude that the rate of tumor growth is not constant throughout the development time, and that the concept that ultraviolet radiation acts by forming tumor cells which immediately adopt a new and

thenceforward constant rate of growth is not consistent with the experimental facts. While this may not constitute an absolute negation of the somatic mutation hypothesis, it seems evident that that hypothesis must be radically modified if it is to fit these data.

III. D. OTHER CARCINOGENS

To what extent is one justified in attributing carcinogenic action to a common mechanism? The present treatment is so general that it seems likely that whatever truth there may be in it, should apply more widely than to the action of a single carcinogenic agent, and it would be of interest to compare the quantitative relationships for carcinogenesis by ultraviolet radiation with those observed with, say, chemical carcinogens.

Although a great many studies have been made with the latter, particularly with polycyclic hydrocarbons, the experiments are of such diverse kinds that there seems little hope of carrying out with the data available an analysis comparable to that which is possible for ultraviolet radiation, but there are some similarities which may be noted. In experiments of Cramer and Stowell (*19*), and of Crabtree (*18*) a regular schedule of application of the carcinogen was maintained until the appearance of tumors, and, for such experiments, plotting percentage incidence of tumors against $\log t_d$ results in a fairly good normal distribution just as in the experiments with ultraviolet radiation. Cramer and Stowell found that when this schedule is discontinued earlier, tumor appearance is delayed and the curves are not so steep, just as in the experiment described in text-figure 8 when exposures to ultraviolet radiation were discontinued. These facts suggest similar temporal relationships when the two kinds of carcinogenic agent are applied in comparable fashion, and may indicate similar relationships with regard to rate of proliferation. Another point of similarity is that with chemical carcinogens, as with ultraviolet radiation, increasing the dosage above a certain value does not decrease tumor development time (*17*), that is, there is a maximum effective dose comparable to D_m in our experiments.

On the other hand, there is a dissimilarity between carcinogenesis by ultraviolet radiation and by some chemical agents. A single dose of the latter, if sufficiently great, may produce tumors; whereas this has not been found for ultraviolet radiation. However, when a chemical carcinogen is applied it remains in the tissue for some time (*1*), whereas with ultraviolet radiation the primary effect ceases with the end of the exposure. Thus, in terms of the theory developed in section III. B. the chemical carcinogen may continue to accelerate the growth rate of tumors for a time after its application. This might account for the discrepancy. The marked influence of interval between doses of ultraviolet radiation suggests that a carcinogen whose action continues for a considerable time, would be particularly effective.

As in the case of chemical carcinogens single doses of ionizing radiation may induce tumors (*22*). Whether this can be attributed to persistent action after the termination of the irradiation is uncertain.

At present there is insufficient experimental data to permit the theory of progressive acceleration of cell proliferation to be generalized to carcinogens other than ultraviolet radiation, but on the other hand there is not adequate evidence for its rejection.

IV. SUMMARY AND CONCLUSIONS

Quantitative studies of carcinogenesis are more complete for ultraviolet radiation than for other agents. The studies with this agent are quantitatively explainable by a simple theory based on the assumption that the proliferation of certain cells—constituting in the beginning a small fraction of the cell population of the normal tissue—is progressively accelerated by successive doses. These cells and their offspring outstrip their fellows and ultimately constitute a tumor.

Other currently accepted theories—including that of somatic mutations—fail, when subjected to analysis, to account for the observed relationships. It seems therefore that the idea of progressive acceleration may be seriously considered until further evidence is obtained—for or against—even though a satisfactory explanation in terms of exact mechanism is not yet apparent. It is, of course, possible that the fit between data and experiment is only accidental, the mechanism of carcinogenesis being too complex to be described by so simple a quantitative relationship. This point of view seems, however, an unprofitable one. It is of course quite possible that the observed relationships are interpretable in terms of a better theory, but to this I have as yet no clue.

It seems improbable that more definitive proof of this theory of carcinogenesis is to be found in terms of experiments of the type which have yielded the present data, since they only permit study of the tumors in late stages. Since the same criticism applies to all studies of cancer induction in higher animals, any theoretical treatment must be regarded as based on extrapolation, as this one admittedly is. It seems likely that the explanation of the mechanism of carcinogenesis may have to come from the study of simpler systems in which cell-proliferation can be more directly studied.

Whatever importance may attach to the present tentative theory, the quantitative relationships found in its development would seem to merit the attention of students of oncology, since so many concepts appear to break down when subjected to analysis in terms of simple quantitative growth relationships. Only through eliminating untenable hypotheses can we hope to arrive at a satisfactory answer.

V. Acknowledgments

A number of people have given me valuable criticisms, either in the early development of the ideas discussed herein, or later on when the work reached manuscript form. I take this opportunity to express my gratitude for such assistance to P. H. Abelson, R. D. Boche, J. T. Bonner, H. Eyring, F. S. Johnson, J. E. Mayer, A. K. Parpart, L. J. Savage, and H. M. Stommel. Their advice has been in most instances followed, but in justice to their kindness I must assume complete responsibility for the final result.

REFERENCES

(*1*) Berenblum, I., and Schoental, R.: The rate of disappearance of 3:4-benzpyrene from the mouse after subcutaneous and intraperitoneal injection. Biochem. Jour. 36: 92–97, (1942).

(*2*) Blum, H. F.: Wavelength dependance of tumor induction by ultra-violet radiation. J. Nat. Cancer Inst. 3: 533–537, (1943).

(*3*) ———: Effect of intensity on tumor induction by ultraviolet radiation. J. Nat. Cancer Inst. 3: 539–543, (1943).

(*4*) ———: Some fundamental aspects of tumor development illustrated by studies with ultraviolet radiation. J. Nat. Cancer Inst. 3: 569–581, (1943).

(*5*) ———: Accuracy and reproducibility in induction of tumors with ultraviolet radiation. J. Nat. Cancer Inst. 4: 75–79, (1943).

(*6*) ———: Growth rate and development of tumors induced with ultraviolet radiation. J. Nat. Cancer Inst. 4: 559–569, (1944).

(*7*) ———: Sunlight as a causal factor in cancer of the skin of man. J. Nat. Cancer Inst. 9: 247–258, (1948).

(*8*) ———: A quantitative description of carcinogenesis by ultraviolet radiation. Cancer Research 9: 545, (1949).

(*9*) ———: Action spectra and absorption spectra. *In* Methods of Biophysical Research, edited by F. M. Uber. New York, Interscience Publ., (1950).

(*10*) Blum, H. F., Eicher, M., and Terus, W. S.: Evaluation of protective measures against sunburn. Am. J. Physiol. 146: 118–125, (1946).

(*11*) Blum, H. F., Grady, H. G., and Kirby-Smith, J. S.: Limits of accuracy in experimental carcinogenesis as exemplified by tumor induction with ultraviolet radiation. J. Nat. Cancer Inst. 3: 83–89, (1942).

(*12*) ———: Relationships between dosage and rate of tumor induction by ultraviolet radiation. J. Nat. Cancer Inst. 3: 91–97, (1942).

(*13*) ———: Effect of ultraviolet radiation on body weight of mice. Am. J. Physiol. 138: 378–384, (1943).

(*14*) Blum, H. F., Kirby-Smith, J. S., and Grady, H. G.: Quantitative induction of tumors in mice with ultraviolet radiation. J. Nat. Cancer Inst. 2: 259–268, (1941).

(*15*) Blum, H. F. and Terus, W. S.: The erythemal threshold for sunburn. Am. J. Physiol. 146: 107–117, (1946).

(*16*) Blum, H. F., Loos, G. M., Price, J. P., and Robinson, J. C.: Enhancement by "visible" light of recovery from ultraviolet irradiation in animal cells, Nature 164: 1011, (1949).

(*17*) Bryan, W. R., and Shimkin, M. B.: Quantitative analysis of dose-response data obtained with three carcinogenic hydrocarbons in strain C3H male mice. J. Nat. Cancer Inst. 3: 503–531, (1943).

(*18*) Crabtree, H. G.: Influence of bromobenzene on the induction of skin tumors by 3, 4-benzpyrene. Cancer Research 4: 688–693, (1944).

(*19*) Cramer, W., and Stowell, R. E.: On the quantitative evaluation of experimental skin carcinogenesis by Methylcholanthrene. The factors of dosage, time, spacing of application, and the multiplicity of the carcinogenic response. Cancer Research 3: 668–681, (1943).

(*20*) Grady, H. G., Blum, H. F., and Kirby-Smith, J. S.: Pathology of tumors of the external ear in mice induced by ultraviolet radiation. J. Nat. Cancer Inst. 2: 269–276, (1941).

(*21*) ———: Types of tumor induced by ultraviolet radiation and factors influencing their relative incidence. J. Nat. Cancer Inst. 3: 371–378, (1943).

(*22*) Henshaw, P. S., Riley, E. F., and Stapleton, G. E.: The biologic effects of pile radiations. Radiology 49: 349–359, (1947).

(*23*) Kirby-Smith, J. S., Blum, H. F., and Grady, H. G.: Penetration of ultraviolet radiation into skin, as a factor in carcinogenesis. J. Nat. Cancer Inst. 2: 403–442, (1942).

(24) LAVIK, P. S., MOORE, P. R., RUSCH, H. P., and BAUMANN, C. A.: Some additive effects of carcinogenic hydrocarbons. Cancer Research 2: 189–192, (1942).

(25) RHOADS, C. P.: Neoplastic abnormal growth. *In* The Chemistry and Physiology of Growth, A. K. Parpart ed., Princeton University Press, (1949).

(26) RUSCH, H. P., and KLINE, B. E.: The influence of a rest period on the carcinogenicity of ultraviolet irradiation applied in interrupted doses. Cancer Research 6: 486, (1946).

(27) ———: The effect of interrupted application of carcinogens on the formation of neoplasms. Cancer Research 9: 545, (1949).

922686—51——8

PULMONARY CARCINOMA REVEALED AT NECROPSY, WITH REFERENCE TO INCREASING INCIDENCE IN THE LOS ANGELES COUNTY HOSPITAL[1,2]

PAUL E. STEINER, M. D., E. M. BUTT, M. D., and HUGH A. EDMONDSON, M. D., *Department of Pathology, The University of Chicago, Chicago, Ill., the Los Angeles County Hospital, and the Department of Pathology, University of Southern California School of Medicine, Los Angeles, Calif.*

Primary carcinoma of the lung is now known to be among the commonest types of cancer (*1*), ranking third to fifth in most necropsy series (*2*), and having in the United States a recognized mortality in 1945 of 11,394 (*3*). While its high incidence is widely accepted, the more important problem of whether it is increasing remains unsolved. Most authors agree that it is increasing, as is all cancer, due to aging of the population. However, disagreement still obtains in regard to the important problem of whether lung cancer is increasing disproportionately to cancer of general or other major types.

It is possible that agreement on this question will never be achieved, because of real local or regional differences in incidence. If additional lung cancers are being induced in considerable numbers by inhalation of carcinogens other than the few now recognized as being so caused (*4, 5, 6*), then regional differences in incidence would be expected. Research on incidence, etiology, and preventive measures in this event should proceed on a regional basis. Each community should be separately studied. If true differences in incidence are established, the causes can be sought in the environments. At the same time, some discrepancies on incidence reported in the literature would be explained. The question of increase is, then, of more than academic interest.

In a previous study by one of us (*2*), using necropsies at the University of Chicago from 1901 to 1943, it was concluded that while carcinoma of the lung had increased, it had done so very slightly more than cancer in general or than a number of other types of cancer, including carcinoma of the colon and intracranial tumors. It was therefore a surprise to find by similar methods of analysis of the Los Angeles County Hospital necropsies, a great increase in lung cancer disproportionate to that of cancer in

[1] Received for publication September 28, 1950.

[2] This study was made with the encouragement of the Research Committee of the Los Angeles County Hospital Staff.

general or to the same control types of tumor. It is believed that these findings represent a real difference in the two materials, and possibly in the two communities. The latter conclusion, if correct, is of such great importance that it should receive serious consideration, and the data are herewith presented.

ANALYSIS OF DATA

From 1918 to 1946 inclusively, 36,864 consecutive necropsies performed at the Los Angeles County Hospital disclosed 6,023 malignant tumors (16.3 percent). This figure includes carcinomas, sarcomas, miscellaneous malignant tumors, leukemias, and related lymphatic diseases, and all fatal intracranial tumors. With a few exceptions the tumor was the cause of death. If the number of tumors is compared in six consecutive 5-year periods a gradual increase is seen from 11.2 percent of all autopsies, in the first period, to 20.7 percent in the last (table 1). Thus the percentage of all autopsies which malignant tumors comprise has nearly doubled in 30 years in this autopsy population. This, no doubt, reflects the reduction in mortality from infectious and other diseases, with resulting aging of the population and ultimate death of more persons from cancer. A higher percentage of hospital beds is now occupied by patients with cancer. The accelerated increase in percent of necropsies showing cancer, beginning in 1938, is undoubtedly due to the added reduction in mortality from acute infections caused by the introduction of the sulfa drugs in that year and the biological antibiotics soon thereafter.

In the 36,864 necropsies, 508 carcinomas and 3 sarcomas of the lung were found. The incidence of carcinoma of the lung was 1.4 percent in all necropsies, and 8.4 percent in all malignant neoplasms. In numerical rank, this tumor occupied fourth place; in men, it ranked second; and in women, it was eighth.

Carcinoma of the lung showed an even greater increase than cancer as a whole. No case of lung cancer was found in the first 5-year period, when the number of necropsies was small, but 158 were disclosed in the last 4-year period, so that the rate in the latter was 2.3 percent of all necropsies and 11.3 percent of all tumors. The increase in the intervening quinquennia was progressive. Carcinoma of the lung, therefore, increased more than fourfold among the causes of death, and it increased at a more than threefold greater rate than did malignant tumors in general.

The disproportionate increase of lung cancer compared with cancer as a whole is seen graphically in text-figure 1. Here is shown the number of cases of carcinoma of the lung found in consecutive groups of 500 malignant tumors. The increase is from 10 lung cancers in the first 500 cancers, to 66 in the last group, a more than sixfold increase by this method of analysis. The greatest increase occurred between 1939 and 1943.

The time and degree of the increase in lung cancer are also shown in text-figure 2, where the number of such tumors is given for each of 42 consecutive groups of 1,000 necropsies performed between 1918 and 1949. There was a gradual increase through the 32 years. An acceleration in the rise

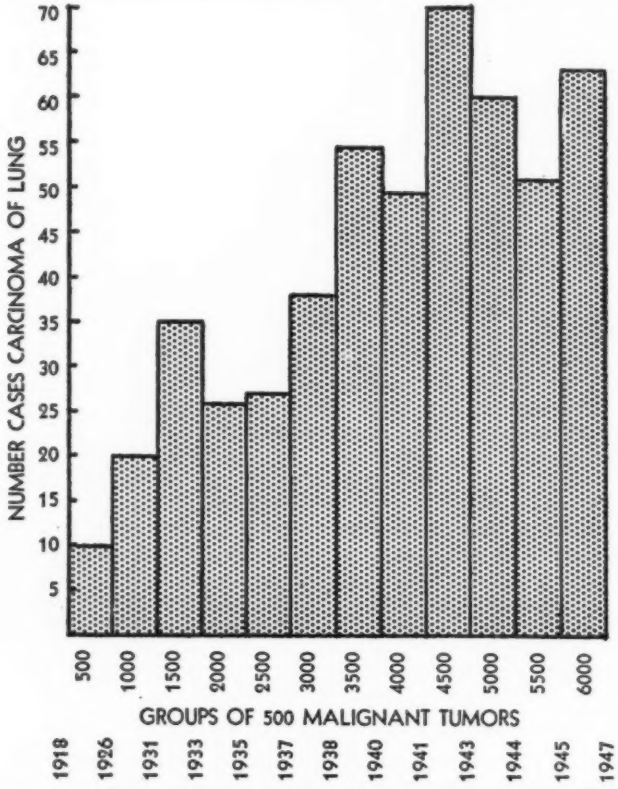
TABLE 1.—*Incidence of carcinoma of the lung by quinquennia in relation to all necropsies, all malignant tumors, and four control tumors*

[Los Angeles County Hospital, 1918–46]

Years	Total autopsies	Malignant tumors		Carcinoma of lung			Carcinoma of stomach			Carcinoma of large intestine			Carcinoma of pancreas			Intracranial tumors		
		Total	Percent of all autopsies	Number	Percent of all autopsies	Percent of all tumors	Number	Percent of all autopsies	Percent of all tumors	Number	Percent of all autopsies	Percent of all tumors	Number	Percent of all autopsies	Percent of all tumors	Number	Percent of all autopsies	Percent of all tumors
1918–22	1, 176	132	11. 2	0	0	0	20	1. 7	15. 2	13	1. 1	9. 8	9	0. 8	6. 8	6	0. 5	4. 5
1923–27	3, 496	493	14. 1	21	. 6	4. 3	65	1. 9	13. 2	42	1. 2	8. 5	22	. 6	4. 5	28	. 8	5. 7
1928–32	5, 055	659	13. 0	29	. 6	4. 4	81	1. 6	12. 3	59	1. 2	9. 0	28	. 6	4. 2	70	1. 4	10. 6
1933–37	9, 647	1, 433	14. 8	83	. 9	5. 8	188	1. 9	13. 1	175	1. 8	12. 2	58	. 6	4. 0	93	1. 0	6. 5
1938–42	10, 741	1, 908	17. 8	212	2. 0	11. 1	246	2. 3	12. 9	189	1. 8	9. 9	98	. 9	5. 1	89	. 8	4. 7
1943–46 [1]	6, 749	1, 398	20. 7	158	2. 3	11. 3	126	1. 9	9. 0	150	2. 2	10. 7	68	1. 0	4. 9	83	1. 2	5. 9
Total	36, 864	6, 023	16. 3	503	1. 4	8. 4	726	2. 0	12. 1	628	1. 7	10. 4	283	. 8	4. 7	369	1. 0	6. 0

[1] 4 years only.

was seen in the period 1939 to 1943. This may be explained by a combination of two factors, namely (*a*) a higher necropsy permit rate in clinically diagnosed lung cancers—higher than that seen before or since (table 2)—and (*b*) an increase in lung cancer along with all cancers (table 1), probably due to a considerable reduction in mortality from infections



TEXT-FIGURE 1.—Number of carcinomas of the lung per group of 500 malignant tumors. (Los Angeles County Hospital, 1918 to 1946.)

(sulfa drugs and antibiotics) and filling of the hospital beds with other diseases, including cancer.

TABLE 2.—*Clinical and necropsy data on carcinoma of the lung*

Period	Number cases diagnosed clinically	Number died	Percent died	Number cases autopsied	Percent autopsies in clinical diagnoses	Percent autopsies in deaths
1918–27	94	63	65	21	22. 3	33. 3
1928–37	527	279	53	112	21. 3	40. 1
1938–45	1, 238	658	53	312	25. 2	47. 4
1946–48	724	361	50	138	19. 1	38. 2

The incidence of lung cancer also increased according to a third method of analysis, namely in proportion to hospital admissions. In table 3 a fourfold increase in the percentage of lung cancer as encountered at necropsy is seen in relation to patients admitted to the hospital between the years 1918 and 1946. In contrast, the total cancer necropsy rate showed a considerable but lesser increase. Expressed in terms of hospital admissions, the necropsy rate for lung cancer increased more than twice as much as did that for all cancers combined, confirming the findings in the entire necropsied population.

TABLE 3.—*Carcinoma of the lung and all malignant tumors in relation to hospital admissions.*

Years	Clinical data			Necropsy data			
	Total hospital admissions	All deaths		All malignant tumors		Carcinoma of lung	
		Number	Percent of admissions	Number	Percent of admissions	Number	Percent of admissions
1918–22	69, 999	9, 260	13. 2	132	0. 19	0	0.
1923–27	110, 639	12, 011	10. 9	493	. 45	21	. 019
1928–32	160, 244	16, 164	10. 1	659	. 41	29	. 018
1933–37	281, 717	23, 779	8. 4	1, 433	. 51	83	. 029
1938–42	256, 201	23, 825	9. 3	1, 908	. 74	212	. 081
1943–46 [1]	221, 199	22, 071	10. 0	1, 398	. 63	158	. 071

[1] 4 years only.

In summary, the incidence of carcinoma of the lung as encountered in necropsies increased greatly in relation to all causes of death, to all cancers, and to hospital admissions. This increase was more than three times as great as that for cancer in general; it was more than four times as great in relation to hospital admissions and to all necropsies.

CONTROL ANALYSES

The question arises whether this increase in lung cancer is truly disproportionately greater than that for all cancer and other types of malignant neoplasms, or whether the increase is explained by some single factor or combination of factors causing an undue number of pulmonary cancer cases to appear in the necropsied population. The following types of control analyses are made to elucidate this point, and to evaluate the conditions of sampling.

Control types of cancer.—It is desirable, as in the previous study, to compare the behavior of lung cancer through the years with other cancers as controls. Carcinomas of the stomach, large intestine (including rectum), and pancreas, and intracranial tumors were selected for this purpose. Compared with carcinoma of the lung, which ranks as fourth commonest in this series of necropsies, the control tumors were also common, occupying the following relative numerical positions: stomach, first; large intestine, second; pancreas, ninth; intracranial tumors, sixth.

These tumors were selected as controls because (*a*) of comparability with the previous study; (*b*) they are major types of cancer; (*c*) no claims for selective increase are made for them; (*d*) they had a low rate of cure comparable to that of lung cancer during most of the period of years under survey in this socioeconomic group, so that necropsy data are not unbalanced or made unreliable from loss of considerable numbers of cured tumors; (*e*) they are all internal tumors whose difficulty in clinical diagnosis during life and pathological recognition at necropsy were, during the period of survey, about equal to that of lung cancer; (*f*) the sex ratios are similar—two of them (stomach and pancreas) have about the same ratio as lung cancer, and the other two have a higher incidence in females; and (*g*) the mean ages of occurrence are such that one of them (intracranial tumors) occurs earlier and the other three later in life.

None of these control tumors showed an increase like that of lung cancer (table 1). The incidence of gastric cancer in all necropsies remained constant; in relation to all cancers it showed a decrease because the number of cancers was increasing. Carcinoma of the large intestine increased in relation to other causes of death, but the rise was no greater than that of cancer in general. The incidence of carcinoma of the pancreas also paralleled that of cancer as a whole. The incidence of intracranial tumors fluctuated more than the others, but, on the whole, it was also comparable to the total cancer picture. The increase, then, among the types of tumors studied, was unique for lung cancer.

Sex factor.—The incidence of lung cancer in the two sexes is unequal. In these necropsies its ratio in males to females was 406:102, but the ratio corrected for sex inequality in the necropsy population was 1:0.41. The question arises whether a shift during the years in the sex ratio of persons subjected to necropsy examination might have accounted for the increase. A great increase in the proportion of males in the necropsy population would tend to increase the number of lung cancers; a decrease in males would tend to reduce lung cancer. A spot check of the sex ratio in the necropsy population in each of the six 5-year periods shows that the proportion of males remained about the same (table 4). The increase in lung cancer is, therefore, not explainable on the basis of a shift in sex ratio in the necropsies.

TABLE 4.—*Sex ratio in 1,000 autopsies in each 5-year period*

Years studied	Males	Females
	Percent	*Percent*
1918–22	62. 6	37. 4
1925–26	63. 6	36. 4
1929–30	60. 3	39. 7
1934–35	64. 6	35. 4
1939–40	62. 3	36. 7
1944	60. 3	39. 7

This conclusion is substantiated by the behavior of the control tumors (table 1). Carcinomas of the stomach and pancreas, in which the incidence is higher in males than in females, maintained about a constant incidence among all autopsies. In contrast, carcinoma of the large bowel, intracranial tumors, and all tumors combined, in which the incidence was greater in females than males, all showed an increase in the 30-year period. Cancer of the lung, despite its preponderance in males, went against the trend of the controls and increased, lending further support to the idea of a real and selective increase.

Age factor.—An increase through the years in the mean age of persons examined post mortem would tend to increase the number of all cancers and of lung cancers. Persons who are saved from death by acute infectious and other diseases must eventually succumb to other causes, and in some instances this is cancer. Table 5 gives the result of a spot check of the average age in groups of 1,000 necropsies sampled near the center

TABLE 5.—*Average age in 1,000 necropsies in each 5-year period*

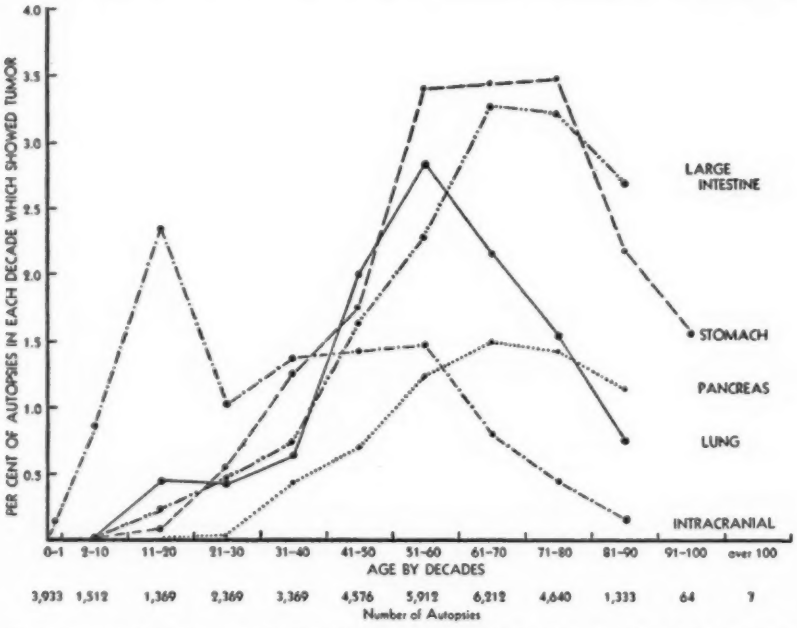
Years studied	Average age
1918–22	40. 1
1925–26	36. 2
1930–31	35. 4
1934–35	43. 0
1939–40	46. 7
1944	53. 1

of each 5-year period. It shows that there was some increase in average age. The disproportionate increase in lung cancer is, however, not explained by this factor. In the necropsy population the death rate from cancer in each decennium was greatest in the decade 61 to 70 years, when it was 25.7 percent. Lung cancer had its peak a decade earlier (text-fig. 2). In contrast, carcinomas of the large intestine and pancreas had their greatest incidence 10 years later than pulmonary cancer, and gastric cancer was maximum 20 years later. If the increase in carcinoma of the lung was due solely to aging of the population, then all cancers, and the control carcinomas of the large bowel, stomach, and pancreas should have increased even more, and intracranial tumors (whose peak incidence was in the second decade) much less. That they did not do so is evidence that the increase in lung cancer was not explainable on this basis.

Factors concerning pathologists.—The allegation has been made that better recognition by pathologists would account for the increase sometimes reported in lung cancer. Better necropsy diagnosis would consist chiefly of the discovery of (*a*) small tumors obscured by other pulmonic disease and formerly missed, (*b*) tumors involving the pleura and mediastinum now recognized as originating in the lung but formerly regarded as primary elsewhere, and (*c*) widespread metastatic tumors in the brain, bones, adrenal glands, kidneys, *etc.*, with a small primary lesion in the

lung formerly not recognized as such but now correctly diagnosed. It is believed that the increase here reported could not be due to these or related factors for the following reasons.

The pathologists who performed or supervised these autopsies were skilled in recognizing primary lung cancer. Before the great increase occurred in 1939–43, they had already recognized nearly 150 cases. Their awareness of the problem of lung cancer and skill in recognizing it



TEXT-FIGURE 2.—Percent of all autopsies performed in each decade which showed cancers of the lung, stomach, pancreas, large intestine, and intracranial tumors. The data are shown as line graphs rather than as bar graphs to facilitate comparison.

was not slowly and progressively acquired over a period of 25 years, as would be the case if the increase were explained by better diagnosis.

This tumor presents no greater difficulty in necropsy recognition than do at least two of the control tumors, namely, carcinoma of the pancreas and intracranial tumors. A small, diffuse, infiltrating glioma, or a small pancreatic cancer mixed with the lesions of chronic pancreatitis and pancreatic atrophy, is fully as difficult to recognize as is a small midzonal or peripheral lung cancer, but the skilled pathologist probably overlooks few of them. These control tumors did not show a corresponding increase.

The great majority of lung cancers fall, grossly and microscopically, within four or five well-known, easily recognized types which have now been standard-textbook and morgue information for several decades. The remainder, which constitute the unusual cases, may be either easy or difficult to diagnose on post-mortem examination. Increase in skill of

diagnosis in the latter category, since it is small, could by no stretch of the imagination account for the great increase in lung cancer here reported.

Neither would changing concepts in nomenclature and classification of pleural, mediastinal, and related types of tumor account for the increase, as is sometimes suggested. The exceptional and rare types of primary lung cancer, plus the other intrathoracic tumors which may simulate lung cancer, plus the widespread metastatic tumors with obscure primary foci, are together numerically so small in proportion to typical lung cancers that they have little influence on statistical studies of the whole problem, and they do not equal and account for the increase here reported.

Better diagnosis by pathologists as the explanation for an increase in lung cancer, especially if the increase is as great as that here reported and in an experience with lung cancer as large as this, deserves to be dropped from further serious consideration. It has unjustifiably confused the problem too long.

The possible role of improvements in clinical diagnosis is another matter.

Clinical factors.—Reported increases in lung cancer are sometimes attributed to greater interest on the part of clinicians or to improvements in clinical diagnosis. In a study based on necropsies, such as this, clinical factors could influence the results by an increase in the percentage of deaths undergoing post-mortem examination or, in the absence of a rising necropsy-permit rate, by a loss of interest in obtaining necropsy permits in other diseases equaling the increased interest in permits in lung cancer cases, overt or suspected.

In these necropsies there is no evidence for the latter, but there is some for the former. The autopsy rate in all hospital deaths increased from 14.5 percent in the first quinquennium (1918–22) to 31.5 in the last (1943–47). Within this doubling of the necropsy-permit rate, the increase in lung cancer might conceivably have been accomplished.

While it is conceded that the rise in necropsy-permit rate might explain the increase in lung cancer, there is strong evidence that it does not. For example, the autopsy-permit rate rose from 37.5 percent in the quinquennium 1933–37 to 44.3 percent during the next 5-year period. Had the former rate prevailed, the number of necropsies performed during the latter period would have been 8,938. Actually 10,741 examinations were made, a difference of 1,803 cases. At the rate of lung cancers then prevailing in necropsies (2.0 percent), only 36 additional cases would have been found, whereas the actual increase was 129 pulmonary cancers. Similar calculations can be made for the other years. The conclusion from this type of evidence is that the rising necropsy-permit rate accounts for only a small part of the observed increase in lung cancer.

Some additional light may be shed on this problem by the data in tables 1 and 3. Whereas the percentage ratio of malignant tumors at necropsy to total hospital admissions tripled between 1918 and 1946 (table 3), the incidence of tumor in all necropsies only doubled (table 1). This indicates that necropsy permits for cancer cases showed relatively less increase than did those for noncancerous diseases. This probably

reflects the relative interest in obtaining permits in these two classes of patients, and it signifies that there was no unusual interest on the part of clinicians in cancer as a whole.

Neither was there any undue interest in obtaining permits for necropsy in clinically diagnosed cases of lung cancer. This is shown in table 2. Here a slight reduction in percent of autopsies made among the cases diagnosed clinically is seen. The increase at necropsy in lung cancer was, therefore, not due to greater interest in obtaining permits in cases clinically diagnosed.

The tumors used as controls do not show the increase exhibited by lung cancer, although there was presumably equally great clinical interest in them. The increase antedates the development of pneumonectomy and lobectomy for therapy of this disease in this hospital—a development which might have attracted referred cases even in this socioeconomic group and with the admission requirements inherent in a charity hospital. Moreover, it might be argued that a vigorous thoracic surgical service would tend to reduce the number of cases seen at necropsy, except in so far as interest in therapeutic failures would result in obtaining more necropsy permits in this class of patient.

Other clinical factors are harder to evaluate. They concern such problems as whether more persons with previously diagnosed or suspected lung cancer were referred to this particular hospital (to be considered later), and whether there was a rising interest on the part of clinicians in retaining diagnosed or suspected cases in the hospital after they were beyond hope of cure. There is no evidence that these factors were functioning to increase the number of lung cancers. The clinicians themselves have no explanation for the increase by these or other factors.

The precipitous increase in lung cancer in the period 1939 to 1943 may be explained in part by the higher permit rate in cases clinically diagnosed (table 2). The rate rose from 21.3 percent in the preceding decade to 25.2 in the period 1938 to 1945, and it dropped to 19.1 percent during the next 3 years. The gradual and greater rise through the years is, however, not accounted for by an increase in the permit rates in clinically diagnosed cases of lung cancer.

In summary, as far as the clinical factors indicate, it must be conceded that while part of the increase in lung cancer might be explained by the rising necropsy-permit rate, it is believed for the reasons given that this or other clinical factors do not explain most of the increase.

Admissions to the hospital.—The patients of this hospital are drawn from the indigent ill of Los Angeles County. It is the only general county hospital for this class of patients, so that there is no competition for lung cancer cases. Its patient load has increased steadily through the years with the increase in population of the county (table 2).

The question arises whether some obscure selective factor might have caused an undue proportion of all lung cancer cases in the county to seek admission or be referred to this particular hospital, despite the stated

nature of its clientele. The possibility is remote that such a factor might be operating, and be large enough to explain the increase, yet remain hidden. The criterion for admission of economic need would tend to prevent a large influx of lung cancer cases, so far as the general population is concerned. As the clinical diagnosis of lung cancer has improved through the years, there has been an increase in the number of cases referred back to the County Hospital from county and other tuberculosis institutions. The number of cases so referred is small in proportion to the lung cancers in the necropsy population, and they do not account for the increase. No large-scale selective referral to this hospital of lung cancer cases is recognized.

Increase in lung cancer by immigration.—The population of Los Angeles County has greatly increased during the period covered in this analysis. This has resulted in increases in the annual number of admissions to the hospital, deaths, and necropsies. Cancer autopsies have been expressed in percent of these factors to correct for the increases. The possibility that the tumor data were influenced by selective immigration of the very young or the very old, or an unequal number of either sex, has already been taken into account in the subsections devoted to age and sex factors; it was concluded that they did not account for the rise in lung cancer.

The possibility must be considered that the immigrants included an undue proportion of persons with pulmonary disease. It is improbable that they had an excess amount of lung cancer. Previously diagnosed cases of lung cancer are not sent by physicians for climato-therapy, nor do they voluntarily seek it—at least not in great numbers. Undiagnosed cases may do so, although the nature and rapid course of the disease probably prevent migration in most instances in this economic group. Undiagnosed cases of chronic pulmonic disease seeking a milder climate would probably contain a relatively large proportion of tuberculous persons. Evidence that selective immigration of pulmonic diseases occurred is not provided by a study of the tuberculosis mortality in the present series of necropsies. In the six quinquennia from 1918 to 1947, the incidence of tuberculosis as a cause of death in the necropsied population fell progressively as follows: 23.2, 14.5, 13.6, 8.8, 7.4, and 5.3 percent. While not all indigent tuberculous patients in the county are cared for at this hospital, there is nothing in these figures to support the idea of selective immigration of persons having pulmonic diseases.

Socioeconomics and occupational exposures.—We are not aware of any surveys reporting an excessive rate (such as is seen in some types of cancer) in lung cancer in social or economic groups of this type. All of the available evidence indicates that within a given area the population suffers equally, with the exception of a few special occupational-exposure groups. If most lung cancer is caused by atmospheric carcinogens, widespread dispersion and nearly equal exposure within a given area would be expected, again excepting the special industrial groups.

To our knowledge no known occupational hazards for lung cancer were unduly represented in this hospital population in either sex. In fact, no

occupation is disproportionately represented except that of the laborer, which is, in final analysis, a very heterogeneous activity and exposure group.

COMMENTS

These data show a great increase in the necropsy incidence of carcinoma of the lung in relation to the entire necropsied population and the total hospital population. The increase was greater than that for cancer in general. A corresponding rise was not shown by the four types of common internal cancers used as controls. The increase was not explained by shifts in age or sex ratio, or by better recognition by pathologists, and probably not by greater clinical interest. Special exposures in occupation were not recognized, and neither were changes seen in the type of case admitted to the hospital, or in selective (as to disease) migration into the county. A small part of the increase might be accounted for by a rising necropsy-permit rate. In the absence of evidence to the contrary, it may be assumed as a tentative working basis that the increase was real, and that it represents the true situation in this socioeconomic group. Since there is ample evidence that the incidence of lung cancer does not vary with socioeconomic conditions *per se*, it is probable that the findings represent the condition existing in the entire population of the county.

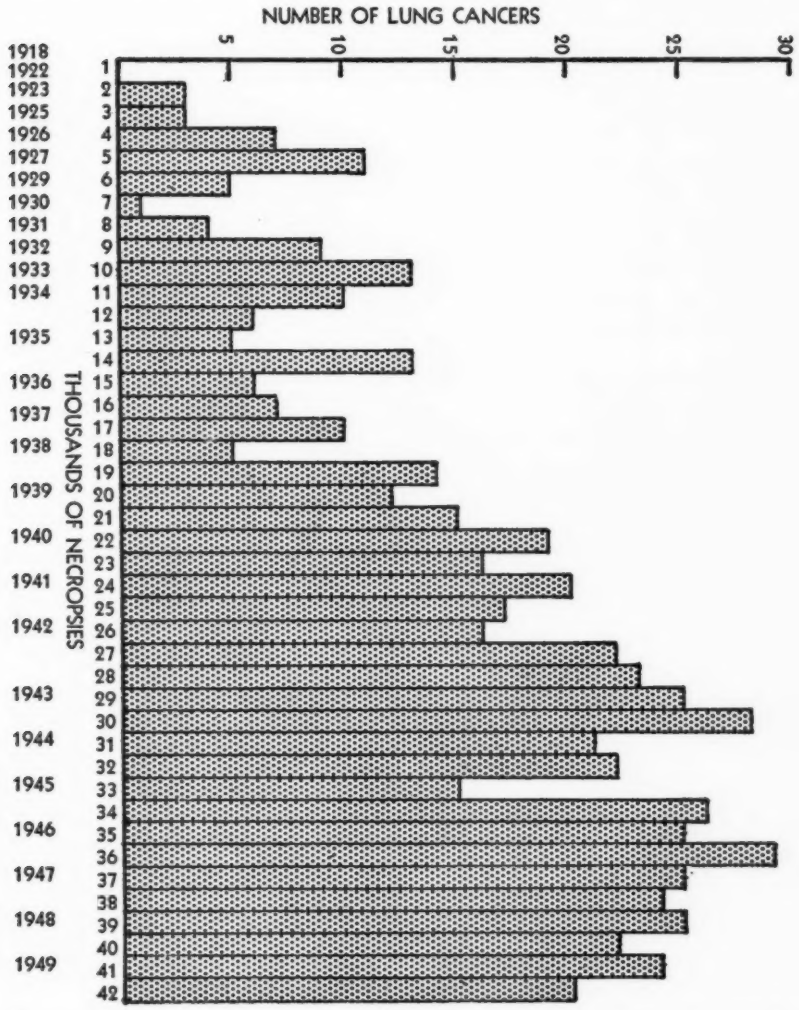
If there has been an increase in the incidence of lung cancer in Los Angeles County as here indicated, what is the cause? On this point one can only speculate. If it were caused by the inhalation of atmospheric carcinogens, the exposures must have antedated the increase by one or more decades, assuming that what is known about tumor-induction time in the case of the hitherto recognized pulmonary carcinogens in man and in experimental animals applies also in this instance (*6*). Neither the high incidence nor the increase is accounted for by the current climatic smog situation, since it is of recent advent, except that previous data on latent period for induction of lung cancer may not be applicable. Neither, however, is smog exonerated; it should be judged as carcinogen or noncarcinogen on its own merits, as may be revealed by future investigations.

A small part of the high incidence of lung cancer here reported, but not the increase, may be explained by the inclusion in this series of a number of Mexican women, who exhibit a high incidence of pulmonary carcinoma. This observation, not previously recognized to our knowledge, will be dealt with in detail in a forthcoming monograph on the racial aspects of the tumors in this necropsy population. For present purposes it may be stated that in Mexicans the incidence of lung cancer was as high in women as in men, and that the incidence in the latter equaled that in Caucasoid men. In Negroids, the incidence of lung cancer was approximately the same as in the Caucasoids. Neither the high incidence nor the increase can be attributed to migration of Negroids into the county.

Regardless of cause, the fact remains that in the last period reported upon in table 1 (1943–46), the incidence of cancer of the lung exceeded that of the stomach. It was the commonest cancer during this period. In the entire 29 years, however, its relative numerical rank was fourth;

it was exceeded by cancers of the stomach, the large intestine, and the malignant lymphatic diseases. It is therefore a major cancer problem.

A problem of great concern is whether the increase is continuing. On



TEXT-FIGURE 3.—Number of primary carcinomas of the lung per thousand necropsies. (Los Angeles County Hospital, 1918 to 1949.)

this point the data are equivocal at present. Text-figure 3 shows no increase between 1943 and 1949, but this period is too short on which to base conclusions. The next decade should supply data adequate for sounder judgment on this point.

Regardless of whether or not the increase in lung cancer is continuing, or even whether it has increased at all, its present level is so high that it constitutes a major public health problem. This challenge should be met by appropriate clinical, experimental, and statistical studies.

SUMMARY AND CONCLUSIONS

In necropsies performed from 1918 to 1949 at the Los Angeles County Hospital, carcinoma of the lung ranked fourth among malignant tumors, and in one recent 4-year period (1943–46), it was first. The incidence of this tumor showed a progressive increase by quinquennia. The increase was more than fourfold in relation to all necropsies, threefold to all cancers, and fourfold to hospital admissions.

Four other types of common tumors, used as controls, namely carcinomas of the stomach, large intestine, and pancreas, and intracranial tumors, did not show a similar increase. The increase was not explained by change in sex ratio or age of persons subjected to necropsy, by increased skill in diagnosis or changes in classification by pathologists, or by immigration, hospital admission, socioeconomic, or occupational factors. A small part of the increase might be due to a rising total necropsy-permit rate, and a small accentuation of the increase in one quinquennium may be explained by a temporarily higher necropsy-permit rate in clinically diagnosed cases of lung cancer.

It is probable that most of the increase in lung cancer was selective, real, absolute, and disproportionately greater than the increase in cancer in general. It is also probable that the increase represents existing conditions in this regard in this socioeconomic group, if not of the entire county population. The causes for the increase are not apparent.

Regardless of whether or not the increase was real, absolute, and disproportionate to all cancer, or whether it is still continuing, the present level is so high as to make lung cancer a public health problem of major concern and investigation in the area.

REFERENCES

(*1*) Dorn, H. F.: The incidence and prevalence of cancer of the lung. Pub. Health Rep. 58:1265–1271 (1943).

(*2*) Steiner, P. E.: Incidence of primary carcinoma of the lung with special reference to its increase. Arch. Path. 37:185–195 (1944).

(*3*) Vital Statistics—Special reports. Deaths and death rates for each cause, United States, 1943–1945. National Summaries 27 (No. 2): 14, May 16, 1947. Federal Security Agency, U. S. Public Health Service, National Office of Vital Statistics, Washington, D. C.

(*4*) Kennaway, E. L., and Kennaway, N. M.: A further study of the incidence of cancer of the lung and larynx. Brit. J. Cancer 1:260–298 (1947).

(*5*) Steiner, P. E., Stanger, D. W., and Bolyard, M. N.: A search for carcinogenic substances in carcinomatous human lungs. Arch. Path. 43:590–601 (1947).

(*6*) Hueper, W. C.: Occupational tumors and allied diseases. Charles C. Thomas, Springfield, Ill. (1942).

EFFECTS OF ROENTGEN RAYS ON CANCER. I. DIRECT MICROSCOPIC OBSERVATIONS ON LIVING INTRAOCULAR TRANSPLANTS OF FROG CARCINOMA [1]

BALDUIN LUCKÉ, M. D., and H. G. SCHLUMBERGER, M. D.,[2] *Department of Pathology, School of Medicine, Univeristy of Pennsylvania, Philadelphia 4, Pa., Department of Pathology, Ohio State University, Columbus 10, Ohio, and the Marine Biological Laboratory, Woods Hole, Mass.*

The experiments reported in this paper constitute a new approach to the study of Roentgen ray effects on cancer, namely, direct microscopic examination of living tumors growing in the anterior chamber of the eye. By this method it is possible to follow the course of events step by step. The observations can be supplemented with serial photographs, which serve as permanent records and are particularly valuable for an objective comparison of the rates of change. The method has the advantage of simplicity; no elaborate operations are required. The anterior chamber of the eye is a natural cavity; the transplanted tumor within it can be viewed through the thin, nonvascular, transparent cornea, using the slit microscope or similar device.

Different kinds of neoplasms from several animal species can be employed for such studies as this. For the present experiments, the renal carcinoma of the leopard frog (*Rana pipiens*) was selected because:

(*a*) This tumor is an adenocarcinoma, and so is representative of the most common variety of cancer in man.

(*b*) It is readily transplantable to the anterior chamber of the eye, where it grows in a distinctive and uniform pattern. The tumor can be maintained by serial transplantation; the incidence of takes rises to 100 percent after the third generation, and consequently an abundance of uniform living material is available.

(*c*) The neoplastic cells are much larger than the cells of corresponding tumors in mammals and birds. The larger size of the cells, and hence of the structural patterns into which they become arranged, makes for greater ease of observation.

---

[1] Received for publication October 13, 1950.

[2] This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

922686—51——4

(*d*) The tumor is sufficiently radiosensitive to be affected by doses of Roentgen rays that do not significantly damage the surrounding normal tissues.

(*e*) Injury to the tumor is detectable by such decisive criteria as change in translucency, in pattern of growth, in rate of growth, and in size.

(*f*) Of interest, for these experiments, is the late vascularization of the growing transplants; the size of the latter may increase manyfold before they acquire a vascular stroma; the currents of fluid in the anterior chamber are adequate for the maintenance and multiplication of the neoplastic cells. As a rule, only the coarser projections and the interior of the tumor mass are supplied with a fibrous support and with vessels. Even when the tumor has become large, the recent outgrowths from its surfaces—solid cylinders of cells, tubules, or cysts—are not vascularized. Thus direct effects of irradiation upon the tumor cells may perhaps be separated, to some extent, from indirect effects resulting from vascular or stromal injury.

The present paper deals with: 1) general types of response after effective irradiation; 2) certain aspects of the so-called time-intensity factor, namely, the comparative effects on tumors when irradiated (*a*) with single large doses of Roentgen rays ranging from 2,000 to 6,000 r, and (*b*) with repeated small doses given on alternate days in quantities of from 200 to 1,400 r, until a total of from 3,200 to 15,300 r is reached; and 3) with retransplantation of irradiated tumors.

METHODS

The method for intraocular transplantation and for the subsequent examination of the tumors has been previously described (*1–3*). Using a finely pointed forceps, small pieces of tumor were placed in the anterior chamber of the eye of frogs through an incision at the sclerocorneal junction. Aseptic precautions were taken, and only tumor fragments of approximately equal size were transplanted. To insure uniformity in the number of takes and rate of growth, tumors were used that had been serially transplanted for from 7 to 9 generations. One large intraocular tumor furnished enough material for reinoculation into approximately 10 animals. The transplants were examined microscopically at intervals of 2 to 3 days, and photographed once each week.

The frogs were kept on wet cotton in individual aquarium jars stored in a thermostatically controlled room at 28° C. For irradiation, photography, or direct observation they were transferred to room temperature.

Transplantation, irradiation, and photography were performed while the frogs were under ether anesthesia.[3]

Radiation was supplied by a Chaoul tube delivering a highly filtered beam generated at low voltage.[4] It permits irradiation of a relatively

---

[3] With some precaution it is possible to anesthetize a frog repeatedly; nearly all animals in this series were etherized for photography at weekly intervals for 2 or 3 months, and in addition were anesthetized on alternate days for fractional irradiation.

[4] We wish to express our appreciation to Dr. Eugene Pendergrass for making available the facilities of the Department of Radiology, and to Drs. Edward Neuhauser and Allan Briney for much technical help and advice.

small object at a short distance from the target and delivers a high and uniform dosage (*4*, *5*). The irradiation effect is superficial, thus avoiding injury to the underlying tissue. The etherized frog was wrapped in moist gauze and placed on its side upon an adjustable stage so that the tumor-bearing eye protruded into the opening of a lead plate shielding the animal.

The technical factors were: 50 kilovolts, 4 milliamperes, 0.2 mm. of copper filter, half value layer=2.4 mm. of aluminum. For 2,000 r (measured in air) the delivery rate was 850 r per minute, cone distance 1.5 cm. from surface of the cornea; for 4,000 r it was 3,600 r per minute, the cone in contact with the cornea; for 6,000 r, the rate was 3,000 r per minute, cone distance 3 mm. For all fractional irradiation the delivery rates were 300 r per minute, and the cone distances 3 cm; the other factors were the same as for 2,000 r.

By the methods outlined, a total of 110 intraocular tumors were examined; 31 of these were irradiated with single, relatively large doses, 12 with multiple (fractional) smaller doses, 11 were retransplanted after previous irradiation, and 56 served as controls.

In all tumors of this series the rates and patterns of growth before irradiation were uniform. Following a lag period of 5 to 10 days after transplantation, rapid cell proliferation occurred at the surface of the transplants, which became increasingly translucent and produced buds that gradually elongated and extended into the aqueous humor, forming solid cylinders, tubules, cysts, or coarser papillary projections. Approximately 1 month after inoculation the size of the transplants had increased several fold; they had become firmly attached, and, though actively growing, many of them were not completely vascularized. At this time the irradiation experiments were begun.

The growth characteristics of these well established tumors in the unirradiated control groups are illustrated by serial photographs of a representative transplant (fig. 1). From about the 30th day after transplantation (upper left photograph) growth continued rather slowly for some 3 weeks; thereafter it proceeded more rapidly, perhaps because the tumors had become better vascularized. As the cyst walls became thicker, and papillary projections extended into their lumina as well as onto the surface as new tubules, the tumors appeared more solid. When most of the anterior chamber was filled, the growth increment became smaller and smaller. In the example illustrated, it required 70 days from the beginning of the observations, *i. e.*, 100 days after transplantation, for the tumor to distend the anterior chamber completely. However, the rate of progress, especially in the later stages of growth, varied with individual tumors. Such variation does not concern us here, for in these experiments we are mainly interested in what happens during the more active and more uniform stages of growth.

RESULTS

Before discussing the effects of varying amounts of Roentgen rays on tumors, the types of response that may occur after any effective dosage

will be summarized. This will define the criteria of radiation injury, and at the same time save repetition.

1. General Types of Response to Irradiation

Loss of translucency.—As stated above, new outgrowths from the transplanted tumor are clear and translucent. Irreversible injury to cell structure, whether produced by irradiation or by other means, leads to progressive loss of translucence. Structures, such as tubules or cysts, become cloudy and dull, granules and coarser white flecks appear, and finally the mass becomes opaque. These are perhaps the earliest perceptible effects of irradiation injury. They may sometimes be seen within 12 hours after intensive irradiation; after lighter doses, the changes may not become distinct for several days. Illustrative examples, at different magnifications, are given in figures 3 and 4. It should be emphasized that the changes seen in these photographs, taken 1 week after irradiation, were noted much sooner by direct microscopic examination. As a matter of convenience and for purposes of comparison, photographs were taken at weekly intervals; whereas direct examinations were made more frequently.

Destruction of new outgrowths.—The second sign of irradiation injury, in chronologic order, is shrinkage, fragmentation, and ultimate disappearance of the most actively growing portions of the tumor: *viz*, the cell cylinders, tubules or cysts that extend from its surface. In these experiments such regression occurred later than the changes in translucence. Early indication was seen approximately 3 days after irradiation and often became pronounced within a week. However, sometimes a post irradiation lapse of 2 or more weeks occurred before the first signs of shrinkage of tubules were noted. The intensity of the dose was the most decisive factor in the time interval. Illustrative examples of destruction of tubules or cysts are given in figures 3, 5, and 8.

Retardation in rate of growth.—This is another of the criteria of injury that varied with the intensity and frequency of irradiation. For example, after a single dose of 2,000 r, no retardation in growth rate was perceptible for 7 or more days (fig. 2). By contrast, after a single dose of 4,000 r or of 6,000 r, no further growth could be perceived (figs. 3 and 5). With fractional doses there was no retardation of growth rate when 200 r were given on alternate days, for a total of 3,200 r. The tumors continued to grow at a normal rate (fig. 6). When 1,400 r were given on alternate days no retardation was evident for about 2 weeks, during which 8,400 r had been administered. Thereafter growth was retarded.

Rate of reduction of tumor mass.—Here, again, intensity and frequency of irradiation were the significant factors. Thus, after 2,000 r in a single dose the rate of reduction in mass that followed retardation of growth rate was slow. By the 16th day after irradiation the mass had shrunk slightly, and further shrinkage continued gradually (fig. 2) until recovery set in. Similarly, shrinkage after 4,000 r was relatively slow, as shown in figure 3. After 6,000 r, shrinkage at first was slow but became rapid be-

tween the 36th and 39th days (fig. 5). Fractional irradiation with 1,400 r on alternate days produced no perceptible change in mass for 3 or 4 weeks; thereafter the tumor shrank abruptly (fig. 9).

2. Effects of Single Irradiation

In these experiments 8 tumors received 2,000 r, 19 tumors 4,000 r, and 4 received 6,000 r at a single exposure. The results are summarized in table 1, and will now be taken up in order.

Table 1.—*Single irradiation of intraocular tumors*

Dose	Number of animals	Period of observation (days)	Results
2,000 r	8	64 to 100	Regression began approximately 10 days after irradiation, and slowly continued for about 30 days. Thereafter rapid and continued regrowth of the tumors.
4,000 r	19	52 to 110	Regression began approximately 6 days after irradiation and continued to complete disappearance of the tumors, except in 1 animal in which the tumor recurred.
6,000 r	4	36 to 168	Regression began approximately 6 days after irradiation, and continued to complete disappearance of the tumors.

2,000 r.—The reaction obtained with this dose was biphasic. In all 8 tumors, growth continued at a normal rate for an average of 7 days. Thereafter regression began, at first slowly, then more rapidly, and later, again at a slower pace. Regression continued steadily for approximately 30 days. Then the process was reversed. Renewed growth set in at a greater than normal rate and continued until the anterior chamber became fully distended.

A representative group of photographs (fig. 2) illustrates the course of events. The transition from regressive to progressive change is best seen between the 42d and 49th days, when rapidity of growth is particularly evident. As will be shown below, the regenerated tumors were transplantable, indicating that recovery from the irradiation injury was complete.

4,000 r.—In all the 19 tumors of this group, irradiation promptly inhibited growth and led to gradual regression, which in all but one case became complete.

On the average, slight reduction in mass was evident 6 days after the exposure, and within approximately 2 weeks the tumors had shrunk to less than one-half of their preirradiation size. Regression continued slowly; within about 50 days the tumors had either disappeared completely or had been reduced to fibrous remnants. These changes are shown in figure 3.

One exceptional tumor appeared to recover in a manner similar to that seen in the 2,000 r group, but when transplants of it were made they failed to survive and grow.

6,000 r.—None of the tumors in this group showed any evidence of continued growth after they had been irradiated. Definite signs of regression were apparent within 5 days. Shrinkage progressed rapidly, and by the 50th day only fibrous strands remained. Figure 5 illustrates this course of events.

3. Effect of Multiple (Fractional) Irradiation

In these experiments the frogs were irradiated every other day for 28 to 34 days.

In table 2 is summarized information concerning dosage, number of exposures, total amounts of irradiation, length of observation periods, number of tumor-bearing animals, and results.

Table 2.—*Multiple (fractional) irradiation of intraocular tumors*

Dose (on alternate days)	Number of exposures	Total amount	Period of observation (days)	Number of animals	Results
200 r	16	3, 200 r	36	3	Continued growth of all tumors at normal rate.
400 r	16	6, 400 r	41	2	Continued growth for 24 days. Slight regression after 4,000 r, but most of the tumor mass persisted after 6,400 r (38 days).
900 r	17	15, 300 r	71 to 153	4	Continued growth for 17 to 26 days. Gradual and eventually complete regression after 7,200 to 9,000 r.
1,400 r	14	12, 600 r	68 to 164	3	Continued growth for 16 to 23 days. Abrupt and eventually complete regression after 9,800 to 12,600 r.

200 r on alternate days to a total of 3,200 r.—No irradiation effects of any kind were observed in the 3 tumors of this group. Growth proceeded in typical manner throughout the entire course of irradiation (fig. 6). The tumors remained clear and translucent, and new extensions continued to bud forth. There was, perhaps, a suggestion that rate of growth became somewhat accelerated. The tumors proved readily transplantable after they had received 1,800 r as well as after 3,200 r.

400 r on alternate days to a total of 6,400 r.—In both tumors growth continued at a normal rate for at least 13 days, or until 2,800 r had been received. During the next 2 weeks a slight retardation in rate became evident, although new outgrowths appeared; at this time 4,000 r had been given. From the 28th to the 38th day, slowing of the growth rate became more obvious, and was accompanied by actual reduction in mass, although the majority of the tumors persisted even after they had received the entire 6,400 r (fig. 7). Inspection of the photograph taken at this

time will reveal numerous coarse white granules, an indication that irreversible injury has taken place at least in some parts of the tumor.

900 r on alternate days to a total of 15,300 r.—No irradiation effects were noted in 4 tumors for 17 to 26 days, during which period they had received from 7,200 to 9,000 r. Thereafter, signs of regression became increasingly evident. No new tubules formed; the old tubules fragmented, fused, or disappeared entirely. Irradiation was continued, however, to a total of 15,300 r on the 39th day. The regressive changes became steadily more marked until by the 58th day the main mass of tumor was nearly resorbed; by the 78th day no traces remained (fig. 8).

1,400 r on alternate days to a total of 12,600 r.—In this group of tumors the irradiation effects were somewhat more variable than in any of the preceding series. All 3 tumors grew progressively and in an entirely normal manner for 16 days, when 9,800 r had been received. At this time 1 abruptly began to regress, and disappeared completely within 2 weeks. The second tumor showed no perceptible regression for 23 days, when it had received 12,600 r. Then its growth rate slowed and tubular details became lost, but the cysts remained clear and translucent for another week. Shortly thereafter regression set in abruptly and by the 49th day only a fibrous nodule remained, which gradually was resorbed (fig. 9). The third tumor showed an intermediate response. It tolerated irradiation for 21 days (11,400 r), after which it degenerated rapidly, so that by the 30th day it was reduced to mere traces that eventually disappeared.

4. Retransplantation of Irradiated Intraocular Tumors

As a test for latent injury several irradiated tumors were removed from the anterior chamber, and portions were retransplanted into other frogs. These tumors were selected from 3 groups: 1) in which active regrowth had followed a period of regression—this is the group that had received 2,000 r; 2) in which 1 tumor had not regressed, as had the others in the group, but was seemingly recovering—this is the tumor from the group that had received 4,000 r; 3) in which fractional doses of irradiation had either not inhibited growth at all or had led only to some retardation—these tumors had been irradiated with 200 r and 400 r to a total of 1,800, 3,200 and 6,400 r, respectively.

The arrangement of the experiments and the results are summarized in table 3.

1) *Transplants from a tumor that had received 2,000 r.*—The course of events in the irradiated tumor that furnished the transplants was similar to that of the tumor shown in figure 2. It will be noted that after a relatively long period of regression, active and rapid regrowth set in. The tumor was removed 77 days after irradiation and retransplanted to the eyes of 3 frogs. It grew at normal rate in all. In the representative example shown in figure 10 (right side of photograph) the tumor filled the anterior chamber in 47 days. These transplants were not further irradiated. The growth after retransplantation proves that recovery from irradiation injury was complete.

TABLE 3.—*Retransplantation of irradiated intraocular tumors*

Amount and rate of previous irradiation	Days after irradiation when transplanted	Number of animals inoculated	Period of observation (days)	Results
2,000 r, as single dose..	77	3	58	Normal and continued growth in all 3 tumors.
4,000 r, as single dose..	52	2	39	Transplants did not survive and grow.
1,800 r, as 200 r on alternate days.	1	3	91	Normal and continued growth in 2 tumors for 20 days. They were then irradiated with 1,400 r on alternate days. Slight regression after 8,400 r.
3,200 r, as 200 r on alternate days.	1	2	25	Continued growth in both tumors.
6,400 r, as 400 r on alternate days.	1	1	30	Gradual regression.

2) *Transplants from a tumor that had received 4,000 r.*—It will be recalled that one of the 19 tumors in this group seemingly recovered after a period of regression; all others disintegrated. The exception was striking. The tumor was removed, divided, and transplanted into 2 frogs. These transplants failed to survive and grow, and it thus became evident that recovery from irradiation injury had been incomplete and temporary.

3) *a. Transplants from a tumor that had received 1,800 r in fractional doses on alternate days.*—As shown above, irradiation with 2,000 r at a single exposure led to regressive changes, which began after 1 week, continued for approximately 4 weeks, and were followed by rapid regrowth. When, however, nearly the same quantity (1,800 r) was given in fractional amounts no disturbances of growth resulted (fig. 6). One such tumor was removed and retransplanted into 3 frogs. The transplants became established in all. The appearance of a representative tumor 20 days after transplantation is shown in the upper left photograph of figure 10. This was a translucent mass that had more than doubled in size after transplantation. Irradiation was then started with 1,400 r on alternate days and continued until 8,400 had been given. After 4,200 r no adverse effect was noted; but 32 days after transplantation, when 8,400 r had been administered, there were early signs of regression (fig. 10). This experiment indicates that the previous exposure to Roentgen rays had not injured the tumor, nor had it altered its susceptibility to subsequent irradiation.

b. Transplants from a tumor that had received 3,200 r in fractional doses of 200 r on alternate days.—The absence of regressive changes in the irradiated tumor that was used for retransplantation is shown in figure 6. One day after the last irradiation the apparently healthy tumor was

removed and retransplanted into 2 frogs. Both transplants became established and grew in a normal manner, indicating that 3,200 r had not effected any injury.

c. Transplant of a tumor that had received 6,400 r in fractional doses of 400 r on alternate days.—The tumor that was transplanted is shown in figure 7. At the end of the course of irradiation it had undergone some reduction in size but was still larger than before irradiation. Some portions of the tumor had been severely injured, as was evident from a loss of translucency and the accumulation of opaque granules in the mass. When retransplanted, there was but little new growth and the tumor gradually regressed, indicating that irreversible injury had occurred.

DISCUSSION

Discussion will be limited to certain aspects of the experiments that were particularly well brought out by the present method, namely, the nature and site of the earliest perceptible regression, the rate of regrowth of tumor after incomplete regression, the transplantation experiments, and the significance of the time-intensity factor. For a broader discussion of the histopathology of irradiation, the reader is referred to the publications of Shields Warren (*6, 7*), Bloom (*8*), Failla (*9*), Spear (*10, 11*), and Van der Werff (*12*).

Nature and site of earliest perceptible regression.—The exact manner in which irradiation causes cell damage is not as yet completely known (*10, 13, 14*). One of the mechanisms concerned is no doubt a disturbance of the physicochemical state of the protoplasm, such as flocculation or coagulation of intercellular colloids (*15*). Morphologically, such a disturbance manifests itself as increasing cloudiness, granularity, and opacity of the normally more or less translucent cells. These changes are seen with clarity in the present experiments; after any effective dose of irradiation they are the earliest visible signs of regression. The cell damage induced by Roentgen rays is not specific: it is of the same nature as the pathologic processes of cloudy swelling, granular and fatty degeneration, and intracellular coagulation. The course of such cell degeneration may perhaps best be followed by observations of living tissue cultures (*16, 17, 18*). As previously pointed out, the method of implanting tissue in the aqueous humor of the living eye has certain similarities to the cultivation of tissue *in vitro* (*19*); it also permits early and clear-cut recognition as well as continued observation of cell damage.

The site of the damage, with respect to the tumor as a whole, is of interest. Invariably the earliest indications of degenerations were in the cylindrical or tubular outgrowths that extend from the surface of the mass. These first lost translucency, became opaque, and later shrank, fragmented, and ultimately disappeared. The surface outgrowths are the most actively proliferating portions of the tumor; their early destruction is in agreement with the finding that the cells of the growing tips of green plants are more sensitive to irradiation than those of other regions (*20*). It is also in agreement with numerous observations made on a great variety of tissues, that cells that divide rapidly (as presumably the

922636—51——5

components of the outgrowths do) are more sensitive than are cells that divide slowly or not at all (*6*, *7*, *9*).

These cell cylinders or tubules have, however, an attribute other than active growth: they are completely avascular; their nutrition and metabolic exchange are carried out solely by diffusion. Now it is well known that the effects of irradiation are both direct—upon the cells themselves—and indirect—upon vessels, stroma, and cell environment. It is also an accepted fact that cells in tissue culture are far less sensitive to irradiation than are the corresponding cells within the living body [see, for example, the papers by Spear (*21*), by Doljanski and Goldhaber (*22*), and Bloom's recent monograph (*8*)]. The complete absence of vessels in the outgrowths from the tumors, and the poor vasculature of many tumors at the time irradiation was started, led us to hope that it would be possible partly to separate direct effects upon tumor cells from indirect effects due to vascular injury. In this we were disappointed. We had not considered, first, that the new outgrowths are members of a large community, *i. e.*, the tumor as a whole, which must be considered as a biologic unity (*9*, *12*); and second, that the cells cannot be dissociated from their environment (*8*), *i. e.*, in this instance not only the tumor from which they arise but the aqueous humor into which they extend. Ionization of the intercellular fluids of the tumor, and of the aqueous humor, may perhaps be a factor that leads indirectly to the injury of the avascular outgrowths. Further experiments are needed to test this possibility.[5]

Rate of regrowth after incomplete destruction.—Recurrence of a tumor is an even more complex process than its destruction. The sequence of events as observed in these experiments is characteristic. After a single exposure to 2,000 r (which appears to be a threshold dose) there follows a latent period during which growth-rate is unaffected; thereafter, the rate diminishes, and then, while regressive changes become obvious, the mass shrinks more and more. After a minimum size is reached, the process is quite suddenly reversed, and growth is resumed at a rate exceeding the normal. The sudden resumption of growth at a greatly accelerated rate is striking and is very similar to that found in experiments with some unicellular organisms (*12*).

The main point of interest is not the recurrence of the tumor but its regrowth at a considerably accelerated rate. It is generally accepted that irradiation does not affect all cells of a tumor equally; some cells appear to have a greater ability to cope with injurious factors. Are these cells stimulated to excessively rapid division and growth by breakdown products of destroyed cells? Or are these cells stimulated to increased activity by incomplete irradiation? Regarding the former question, there is evidence that some products of cell destruction are growth stimulating, at least to cells of the same kind. But opinion is divided about the possible stimulating effects of inadequate doses of Roentgen rays. The opposing views may be summed up by citing two recent publications. Van der

[5] For a recent discussion of such ionization effects, reference should be made to the review of Zirkle (*25*) and the monographs of Lea (*14*) and Spear (*11*).

Werff (*12*) states, "I do not see why new-formed ions must necessarily always destroy and why they should not be able to stimulate." Bloom (*8*) found no evidence for a stimulating effect of ionizing radiation, and believes that the supposed stimulation is rather a reparative overcompensation after damage. His view expresses the prevalent hypothesis.

With small fractional doses of irradiation, the process of recovery is very difficult to follow. When, for example, 1,400 r (which as a single dose is below the effective threshold of 2,000 r) is given on alternate days, growth continues for 16 to 23 days; then regression sets in abruptly. During the first period there is probably an interplay between destruction and recovery. The relative rates of these two processes may change as the total amount of irradiation increases, until finally the rate of recovery is greatly exceeded by the rate of destruction.

With a smaller dose of Roentgen rays (200 r on alternate days) no destruction is evident at any time, but the rate of growth of the tumor seems to become somewhat accelerated—an impression rather than a certainty. It would seem profitable to design further experiments to clarify this matter, which is of much theoretical interest.

The significance of the time-intensity factor.—The preceding discussion is closely related to the present subject. The relative effectiveness of Roentgen rays in single or in fractional doses is still disputed. It has long been known that a quantity of Roentgen rays is more destructive in a single dose than in divided (fractional) doses given at intervals (*9*, *11*, *12*). The difference may be accounted for by a number of factors, such as: partial recovery of irradiated cells in the intervals between exposures, and adaptation of the cells that may in time become less sensitive or, as is commonly said, more radio-resistant. These are perhaps the two most significant variables. From the practical point of view there are, however, other considerations. This subject has recently been fully reviewed by Strandqvist (*24*). Here we may content ourselves with pointing out the need for further experimental investigation, and with the usefulness of the present method for such studies.

Our experiments have shown that a single inadequate dose (2,000 r) leads to but partial destruction of the tumor followed by rapid recurrence, whereas single adequate doses (4,000 or 6,000 r) effect complete and permanent destruction. With fractional doses, considerably greater intensities of Roentgen rays are required to destroy the tumor. Thus, 3,200 r given as 200 r on alternate days was entirely ineffective, and 6,400 r given in doses of 400 r on alternate days was only partially effective. As much as 7,200 to 9,000 r was required to destroy the tumor when administered as fractional doses of 900 r on alternate days. Similarly, when 1,400 r was given in this manner, the effective dose was found to be as high as 9,800 to 12,600 r. From these figures it is apparent that the larger the individual fractional dose of Roentgen rays the larger will be the total dose required to bring about destruction of the tumor. This is due in part at least to the lag period that follows irradiation, permitting the administration of a relatively large total number of

roentgens in a series of fractional doses before the effect of the first in the series becomes apparent. The cumulative effect of irradiation may then be manifested by the abrupt onset of regression, as noted in the preceding section.

SUMMARY

The effect of Roentgen rays was studied by direct microscopic examination of living intraocular transplants of frog renal adenocarcinoma. This tumor has proved particularly suitable for the purpose because it can be readily established in the anterior chamber of the frog's eye, where it grows in a distinctive pattern. Details in the form of the tumor and of changes induced by irradiation can be observed with ease through the slit lamp microscope, or a similar device, and can be recorded by photographs.

The effect of Roentgen rays on well-established tumor transplants has been studied: (*a*) upon irradiation with single doses of 2,000, 4,000, and 6,000 r, and (*b*) upon repeated irradiation with small doses on alternate days, using 4 different series with 200, 400, 900, and 1,400 r, respectively, given until a total of from 3,200 to 15,600 r was reached.

Three main types of responses were observed:

1) Increasing granularity and opacity of the neoplastic outgrowths, probably due to flocculation and coagulation of intracellular colloids; shrinkage, fragmentation and ultimate disappearance of the most actively growing, nonvascularized parts of the tumor; progressive diminution in size and reduction of the tumor as a whole to a fibro-hyaline mass, or its complete disappearance. These regressive changes occurred rapidly after a single large dose of 6,000 r, or more gradually after a single dose of 4,000 r. With repeated small doses of 900 or 1,400 r, a total of from 7,200 to 12,600 r was required to effect regression.

2) A typical diphasic response. Gradual cessation of growth and onset of regression accompanied by progressive diminution in size for approximately 4 weeks. Thereafter renewed and rapid regrowth of the neoplastic tissue. Such changes were observed after single doses of 2,000 r. The absence of residual irradiation effects was shown by the transplantability of the tumors at the end of the experiment.

3) No perceptible effects of irradiation. The tumors continued to grow in a normal, or perhaps somewhat accelerated, manner. This was the case when doses of 200 r were given on alternate days for a total of 3,200 r. The absence of any injury to the irradiated tumors was further demonstrated by their continued growth after retransplantation.

This method of studying the effects of irradiation by direct microscopic examination, supplemented by serial photographs, has made it possible to observe in considerable detail the processes of regression. It also has brought experimental confirmation of two concepts widely accepted in radiology: first, that much larger amounts of irradiation are required to produce a certain effect when given in divided (fractional) doses than in a single dose; second, that incomplete regression of a neoplasm due to inadequate irradiation may lead to resumption of growth at an unusually rapid rate.

REFERENCES

(1) LUCKÉ, B., and SCHLUMBERGER, H.: Manner of growth of frog carcinoma, studied by direct microscopic examination of living intraocular transplants. J. Exper. Med. 70: 257–268 (1939).

(2) ———: Effect of temperature on the growth of frog carcinoma. I. Direct microscopic observations on living intraocular transplants. J. Exper. Med. 72: 321–330 (1940).

(3) SCHLUMBERGER, H., and LUCKÉ, B.: Serial intraocular transplantation of frog carcinoma for fourteen generations. Cancer Research 9: 52–60 (1949).

(4) PENDERGRASS, E. P., HODES, P. J., and GARRAHAN, C. J.: Roentgen therapy by the method of Chaoul. Radiology 32: 142–154 (1939).

(5) CHAMBERLAIN, R. H.: Recent advances in contact therapy equipment and usage. Pennsylvania M. J. 53: 359–362 (1950).

(6) WARREN, S.: Effects of radiation on normal tissues. Arch. Path. 34: 443–450; 562–608; 749–787; 917–931; 1070–1084 (1942); 35: 121–139; 304–353 (1943).

(7) ———: Histopathology of radiation lesions. Physiol. Rev. 24: 225–238 (1944).

(8) BLOOM, W.: Histopathology of Irradiation from External and Internal Sources. McGraw-Hill Co., New York (1948).

(9) FAILLA, G.: Biological effects of ionizing radiations. J. Applied Physics 12: 279–295 (1941).

(10) SPEAR, F. G.: Biological effects of penetrating radiations, review. Brit. M. Bull. 4: 2–11 (1946).

(11) ———(Ed.): Certain aspects of radiation on living cells. Brit. J. Radiol., suppl. 1 (1947).

(12) VAN DER WERFF, J. Th.: Biological Reactions Caused by Electric Currents and by X-rays. Elsevier Publishing Co., New York (1948).

(13) RUGH, R.: Effect of ionizing radiation of gametes, embryos and the newborn. Trans. N. Y. Acad. Sc. 2: 55–57 (1949).

(14) LEA, D. E.: Actions of Radiations on Living Cells. Cambridge Univ. Press & Macmillan, New York (1947).

(15) HEILBRUNN, L. V., and MAZIA, D.: The action of radiations on living protoplasm. Chap. 18 in DUGGAR, B. M. (Ed.): Biological Effects of Radiation. McGraw-Hill Co., New York (1936).

(16) LEWIS, W. H.: Degeneration granules and vacuoles in the fibroblasts of chick embryos cultivated *in vitro*. Bull. Johns Hopkins Hosp. 30: 81–91 (1919).

(17) LEVI, G.: Explantation, besonders die Struktur und die biologischen Eigenschaften der *in vitro* gezüchteten Zellen und Gewebe. Ergebn. d. Anat. u. Entwcklngsgesch. 31: 125–707 (1934).

(18) FISCHER, A.: Biology of Tissue Cells. Cambridge Univ. Press, England (1946).

(19) LUCKÉ, B.: Characteristics of frog carcinoma in tissue culture. J. Exper. Med. 70: 269–276 (1939).

(20) JOHNSON, E. L.: Effects of x-rays upon green plants. Chap. 29 in DUGGAR, B. M. (Ed.): Biological Effects of Radiation. McGraw-Hill Co., New York (1936).

(21) SPEAR, F. G.: Tissue culture; its application to radiological research. Brit. J. Radiol. 8: 68–86 (1935).

(22) DOLJANSKI, L., and GOLDHABER, G.: Radiobiological studies on tissue cultures; immediate effect of x-rays on cell outgrowth in cultures of fibroblasts. Growth 6: 235–249 (1942).

(23) ZIRKLE, R. E.: Relationships between chemical and biological effects of ionizing radiations. Radiology 52: 846–855 (1949).

(24) STRANDQVIST, M.: Studien über die kumulative Wirkung der Röntgenstrahlen bei Fraktionierung. Acta Radiol., suppl. 55 (1944).

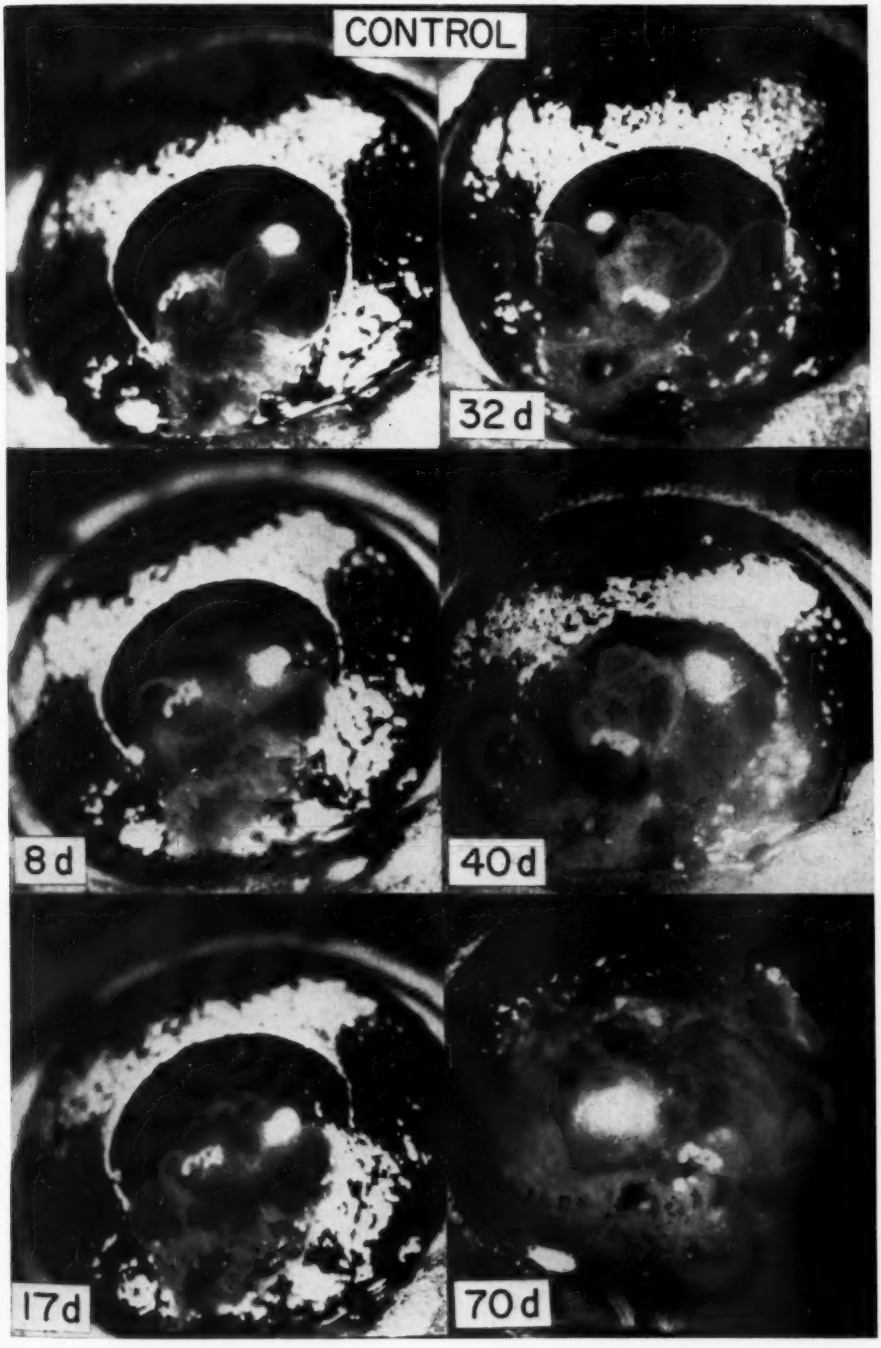
922686—51——6

PLATE 51

FIGURE 1.—A series of photographs of a representative intraocular transplant of frog kidney carcinoma illustrating the rate and pattern of tumor growth in a control animal kept at 28° C.

The unnumbered photograph shows the tumor approximately 1 month after inoculation. It is now well established; the outgrowths are tubules that have become distended with fluid to form cysts. This is the stage at which the tumors in the experimental series were irradiated.

The numbers on the photographs correspond to the days subsequent to the initial observation. Through thickening of the cyst walls, inward budding, and growth of new tubules, solid masses form that eventually fill the anterior chamber. × 9.



922686—51——7

PLATE 52

FIGURE 2.—Effect on the tumor of a single irradiation with 2,000 r.

The photographs reveal gradual and prolonged regression followed by subsequent rapid regrowth of the tumor transplant.

The unnumbered photograph shows an actively growing transplant approximately 1 month after inoculation, and 1 day before it was irradiated. Seven days later it had increased noticeably in mass. Thereafter, early signs of regression set in; by the 16th day the tumor had shrunk slightly; by the 25th day it was considerably reduced; and during the following week it reached its minimal size. Between the 32d and 42d day slight regrowth became evident, and during the next week the mass enlarged rapidly; growth continued until the entire anterior chamber became filled. × 9.

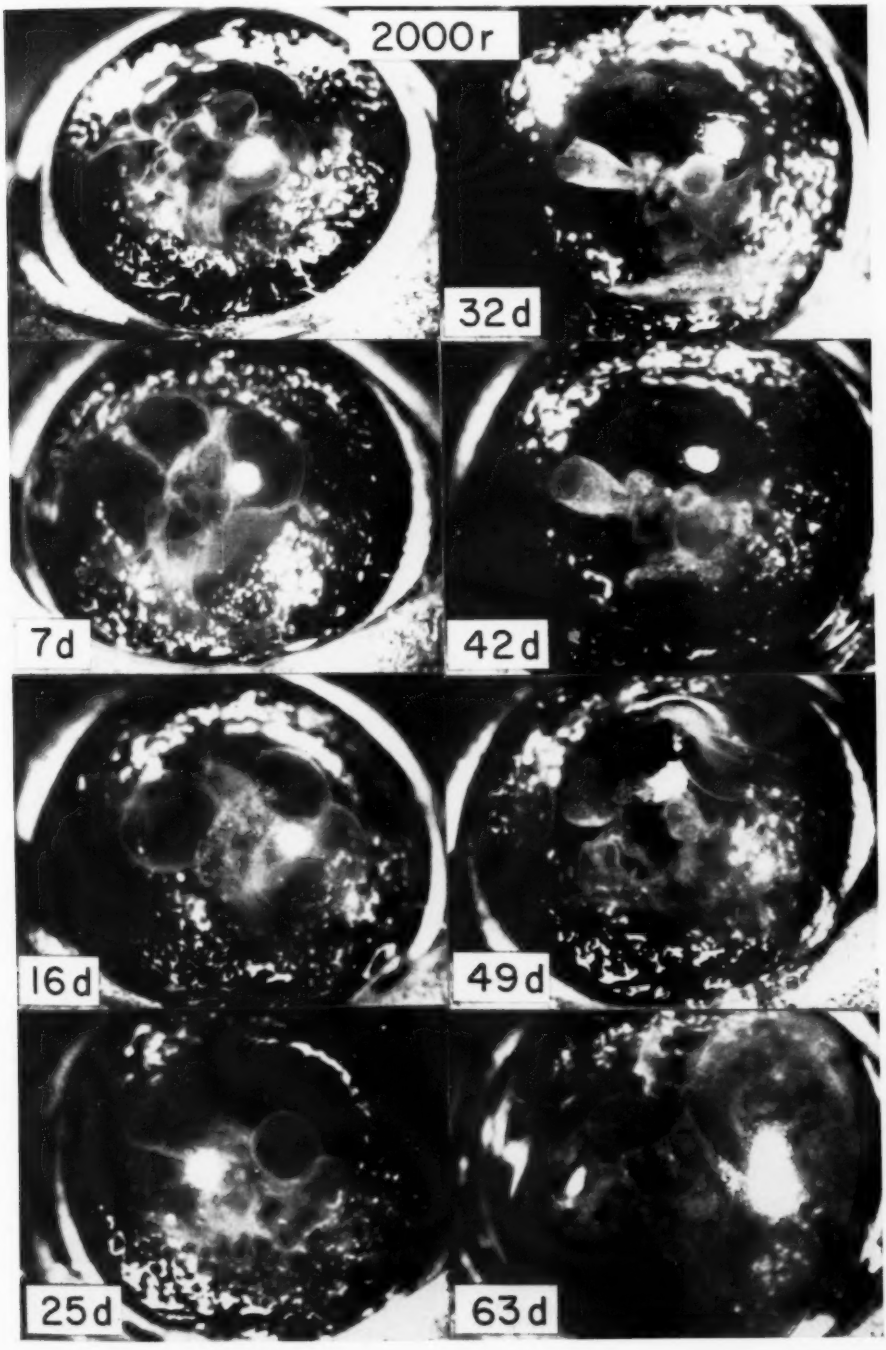

2000r
32d
7d
42d
16d
49d
25d
63d

PLATE 53

FIGURE 3.—Effect on the tumor of a single irradiation with 4,000 r.

The photographs are representative of the gradual and complete regression that occurred in all but 1 of the 19 tumors of this group.

Six days after irradiation the tumor had shrunk slightly, and 8 days later the mass was reduced to less than one-half of its initial size. During the next 5 weeks regression continued slowly until only a vaguely outlined cyst remained. No regrowth occurred.

The photographs on the right were made at the same time as the preceding, but at higher magnification. They illustrate an early sign of regression, namely, increasing cloudiness, opacity, and granularity of the tissue.

Photographs on left at × 9; on right at × 20.

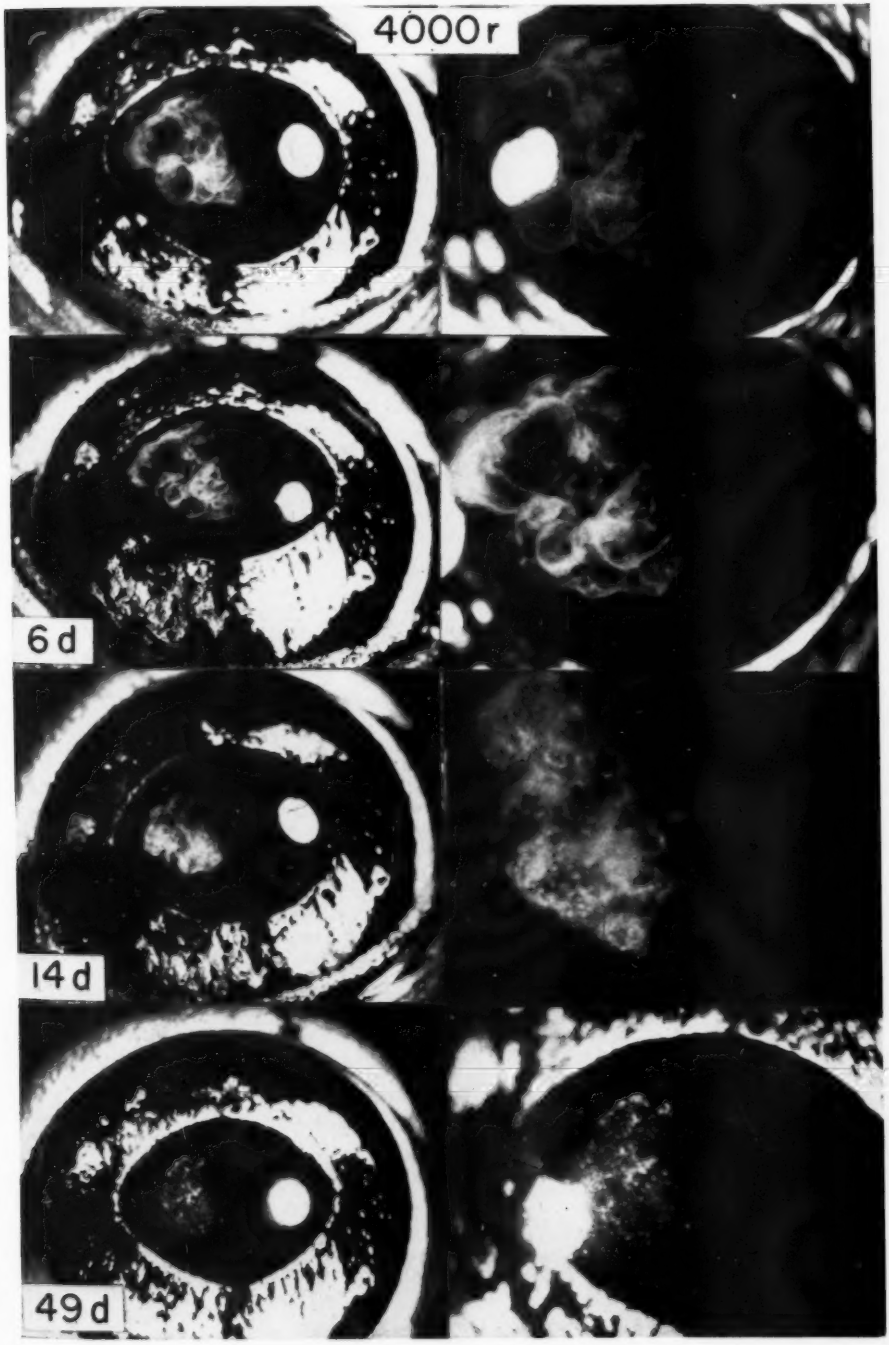

4000r
6d
14d
49d

PLATE 54

FIGURE 4.—Effect on the tumor of a single irradiation with 4,000 r.

The photographs disclose details of the regressive changes as seen at high magnification (× 100). The upper left photograph shows a tubular outgrowth 1 day before irradiation. The walls of the branching tubule are translucent; even the outlines of some individual cells are visible. Seven days after irradiation the tubule had become opaque and shrunken, its branches fused, and the cellular outlines had entirely disappeared. Ten days later all structural detail had vanished and only formless remnants of the tubule remained.

The lower right figure shows at lesser magnification (× 30) the remnants of the entire tumor 44 days after irradiation; it is now contracted to a smooth-surfaced, dense, opaque, fibrous, vascularized mass. Such fibrous remnants sometimes persist for months.

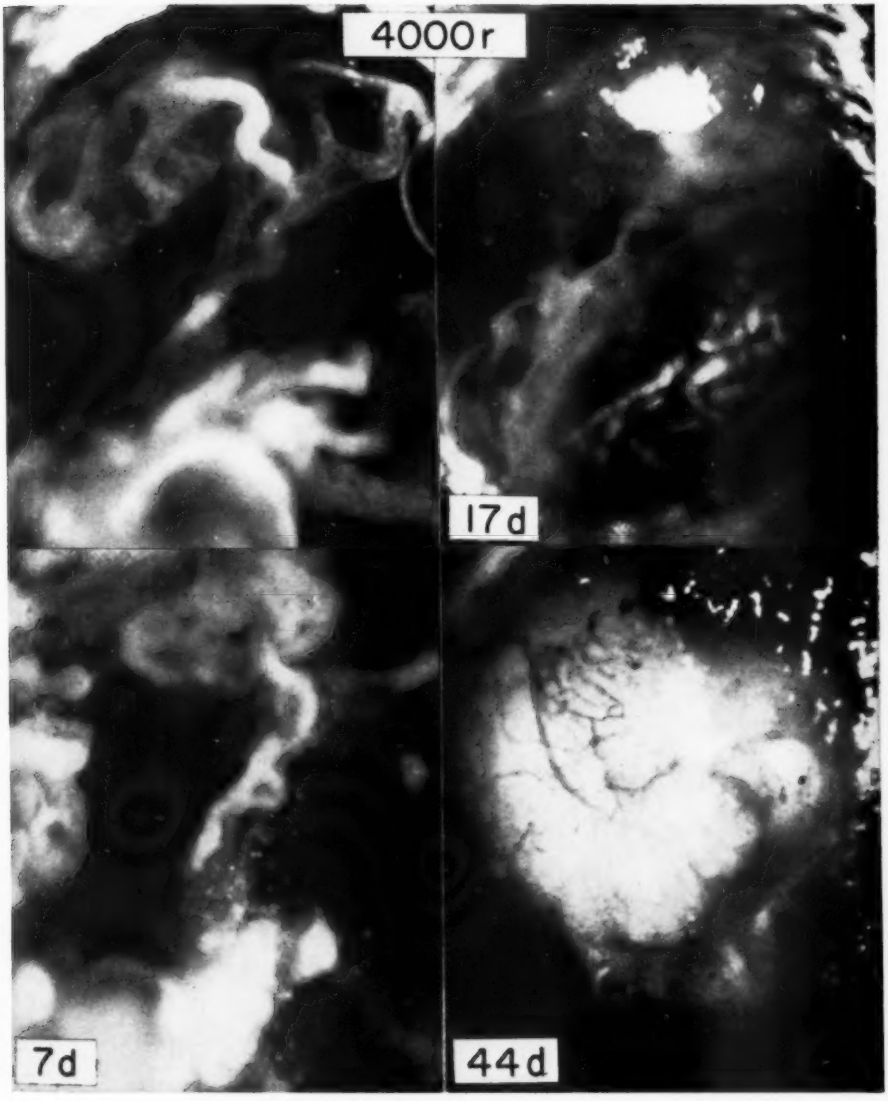
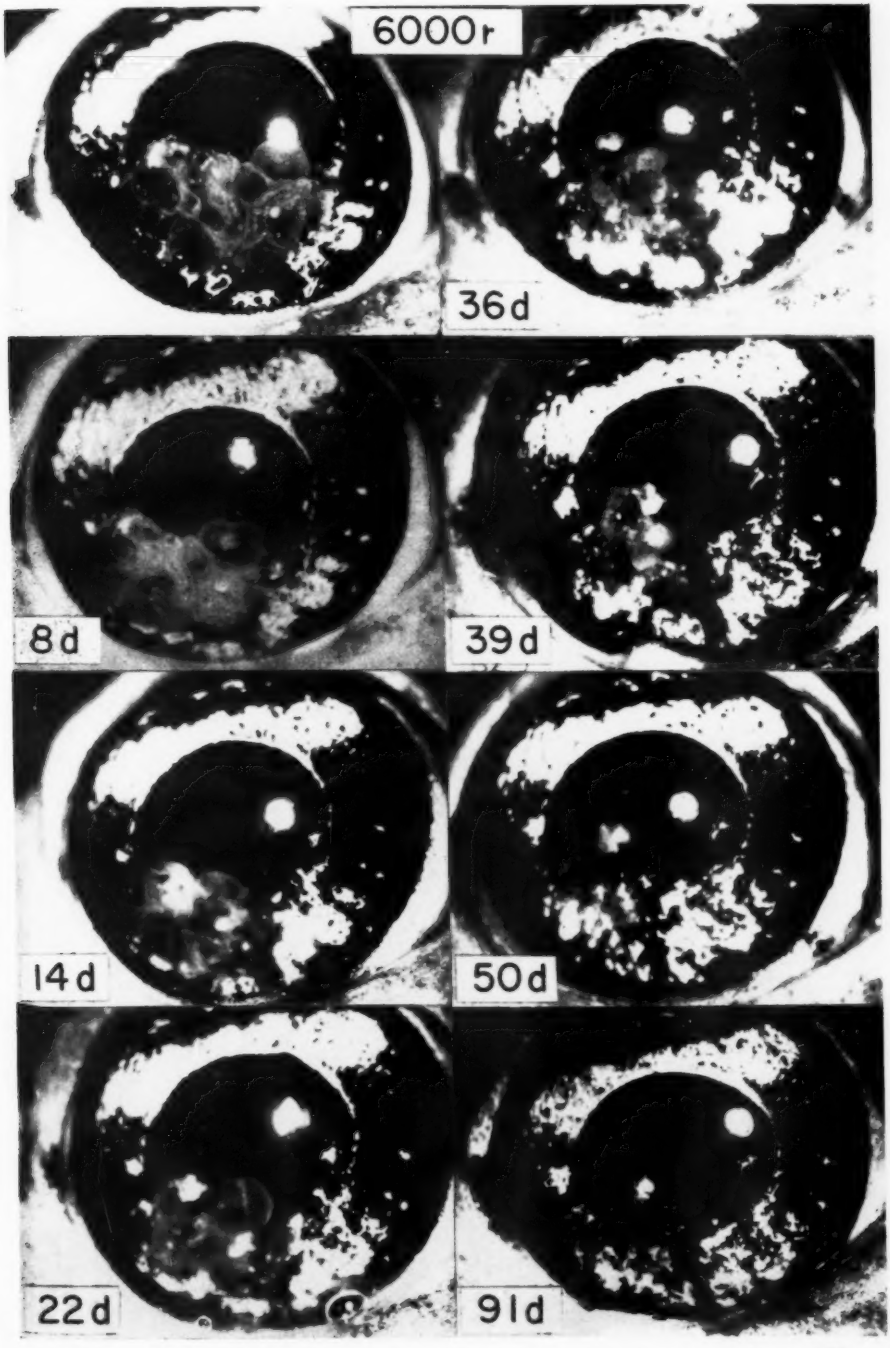

4000r
17d
7d
44d

PLATE 55

FIGURE 5.—Effect on the tumor of a single irradiation with 6,000 r.

The upper left photograph shows a translucent tubulo-cystic tumor on the day before irradiation. Signs of regression, such as increasing opacity, loss of tubular detail and collapse of cysts, were noted within 5 days after irradiation, and by the 8th day were well established. Thereafter the regressive changes continued without interruption. By the 50th day only some fibrous remnants remained; these gradually disappeared, although one small nodule was still present 91 days after irradiation. ×9.



Lucké and Schlumberger

922686—51——8

PLATE 56

FIGURE 6.—Effect on the tumor of fractional irradiation with 200 r on alternate days until a total of 3,200 r had been given.

The upper left photograph shows the tumor 1 day before irradiation. At the bottom of the other photographs are given times after irradiation in days, and total amount received in r. Throughout the experiment, the tumor grew in the manner of the unirradiated control transplants.

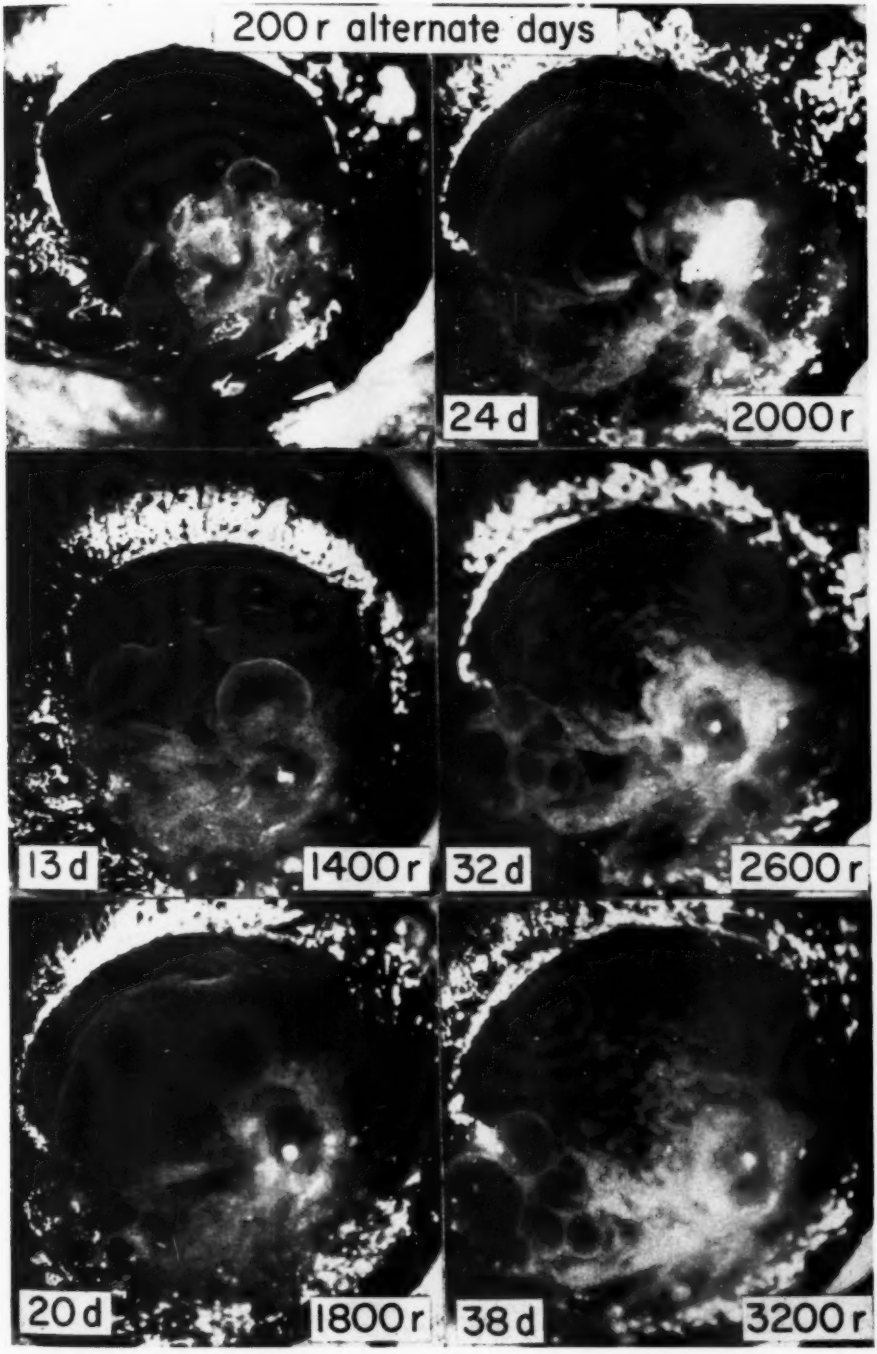

200 r alternate days
24 d
2000 r
13 d
1400 r
32 d
2600 r
20 d
1800 r
38 d
3200 r

PLATE 57

FIGURE 7.—Effect on the tumor of fractional irradiation with 400 r on alternate days until a total of 6,400 r had been given.

The tumor grew at the usual rate for 24 days after irradiation, during which period it had received 4,000 r. At this time signs of regression appeared, but even 14 days later, when it had received a total of 6,400 r, the tumor was still larger in mass than before irradiation. However, numerous coarse white granules in some portions of the tumor indicate foci of degeneration and necrosis. ×9.

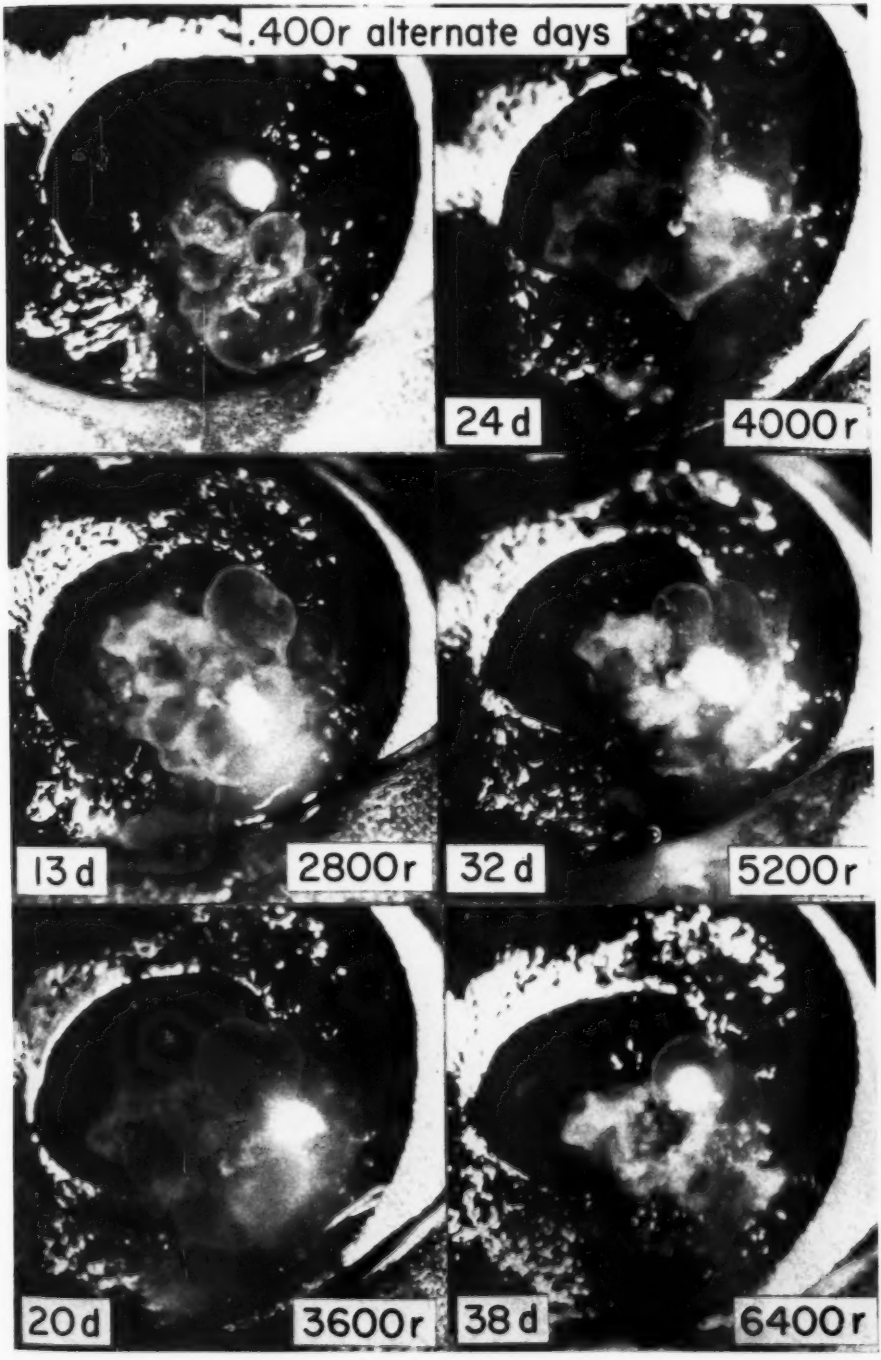

400r alternate days
24 d
4000r
13 d
2800r
32 d
5200r
20 d
3600r
38 d
6400r

PLATE 58

FIGURE 8.—Effect on the tumor of fractional irradiation with 900 r on alternate days until a total of 15,300 r had been given.

The upper left photograph shows the tumor 1 day before it was irradiated. Seven days later, having received 2,700 r, the tumor had increased in mass and developed additional tubules. After another 9 days, when the total amount of radiation had reached 6,300 r, there was still slight evidence of growth, though at a reduced rate. By the 23d day (9,000 r), growth had almost ceased. Thereafter regression began, the tubules became fragmented and increasingly opaque. Irradiation was continued until 15,300 r had been given, on the 39th day. Subsequently, the remnants of the tumor disappeared entirely.

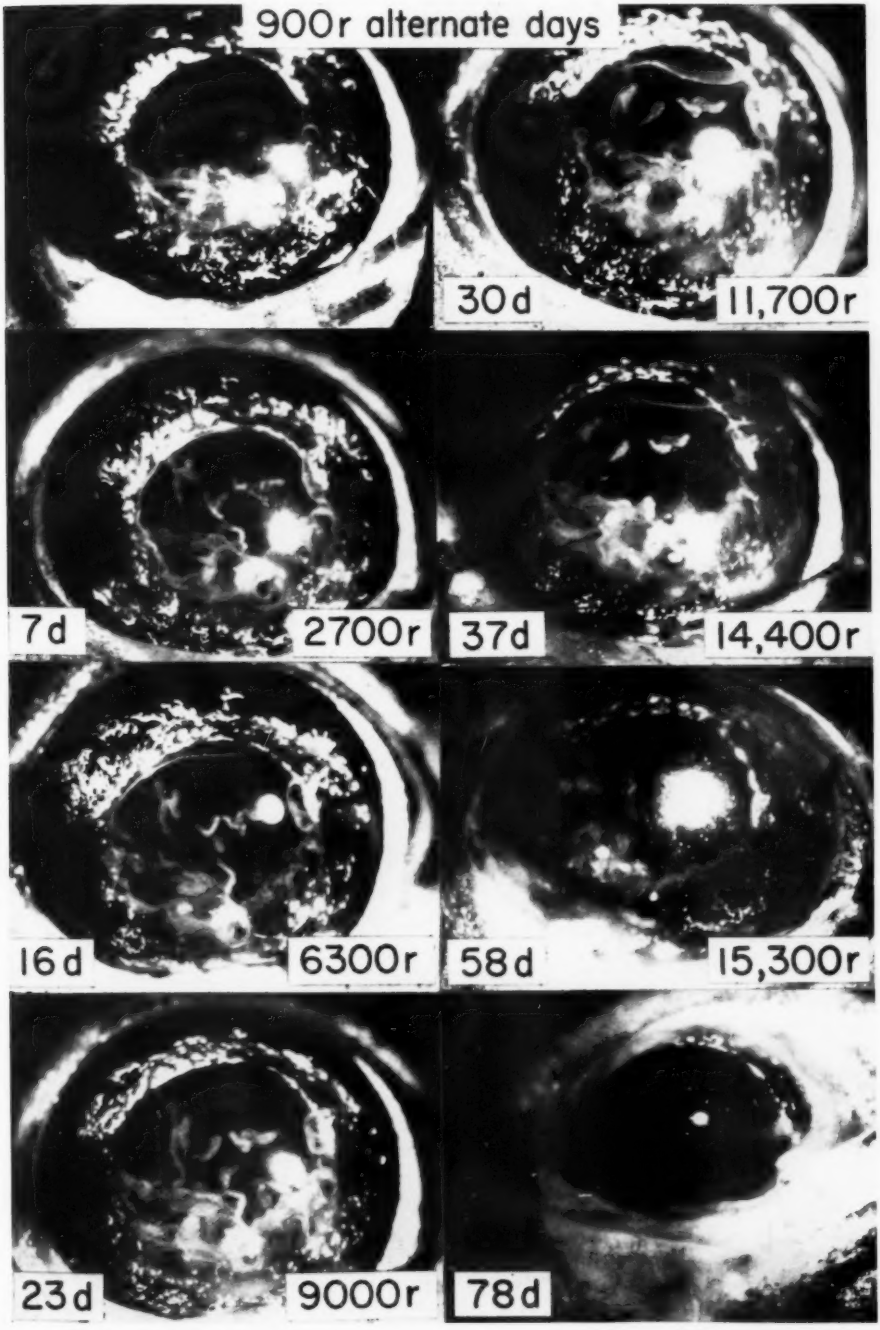

900r alternate days
30d
11,700r
7d
2700r
37d
14,400r
16d
6300r
58d
15,300r
23d
9000r
78d

PLATE 59

FIGURE 9.—Effect on the tumor of fractional irradiation with 1,400 r on alternate days until a total of 12,600 r had been given.

The upper left photograph shows a tubulo-cystic tumor 1 day before irradiation. During the following 7 days, the transplant received 4,200 r, but tubular and cystic growth continued. At the end of the following week, when a total of 8,400 r had been given, the tumor was still increasing in mass and no signs of regression were evident. At the close of the 3d week (11,400 r) there was little change in size and appearance. A final exposure to 1,400 r was given on the 23d day, bringing the total to 12,600 r. Seven days later, tubular detail had been lost, but the cysts were little changed. Within 2 to 3 days, more extensive regression set in, and by the 49th day only a fibrous nodule remained, which gradually disappeared.

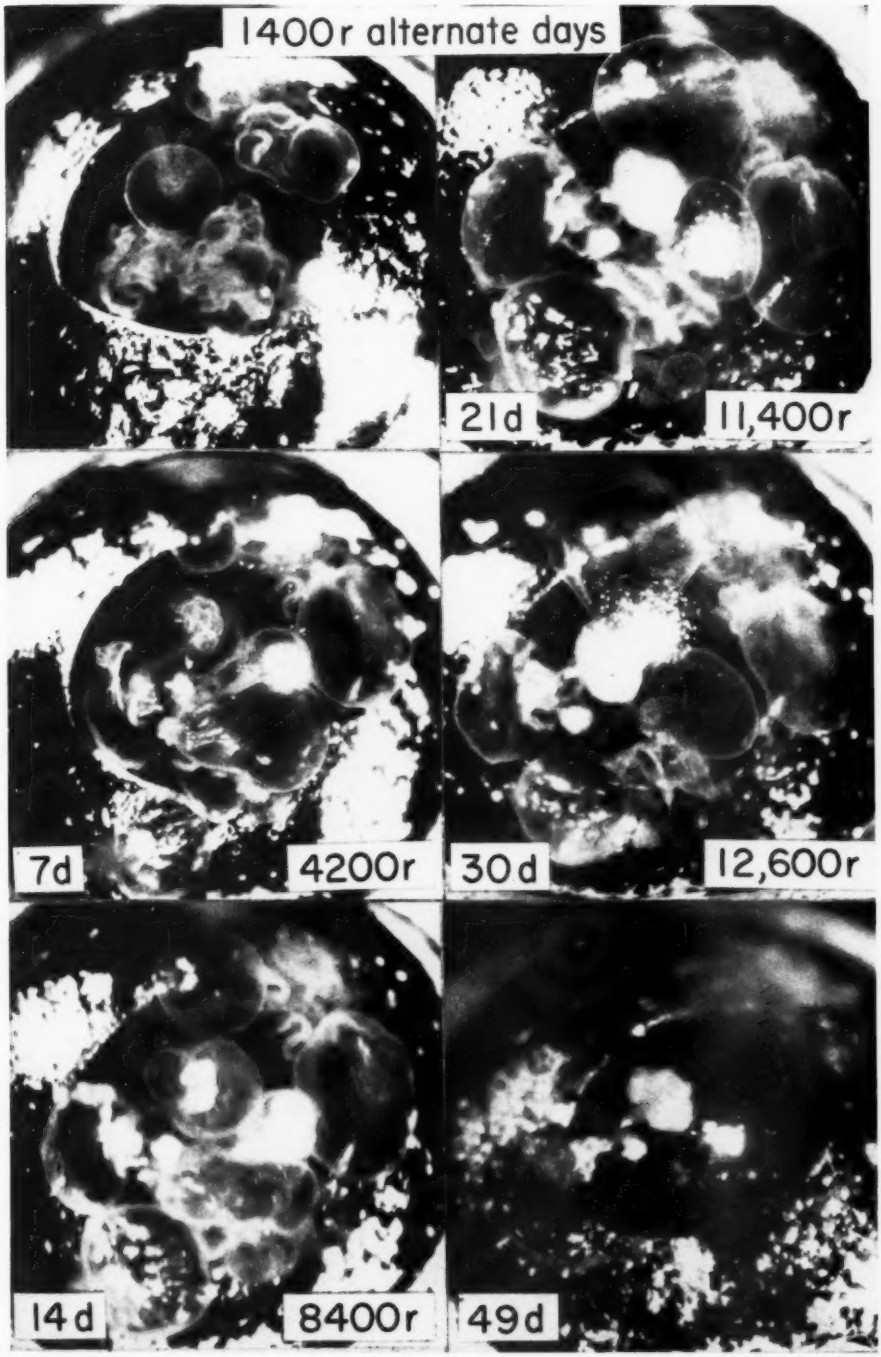
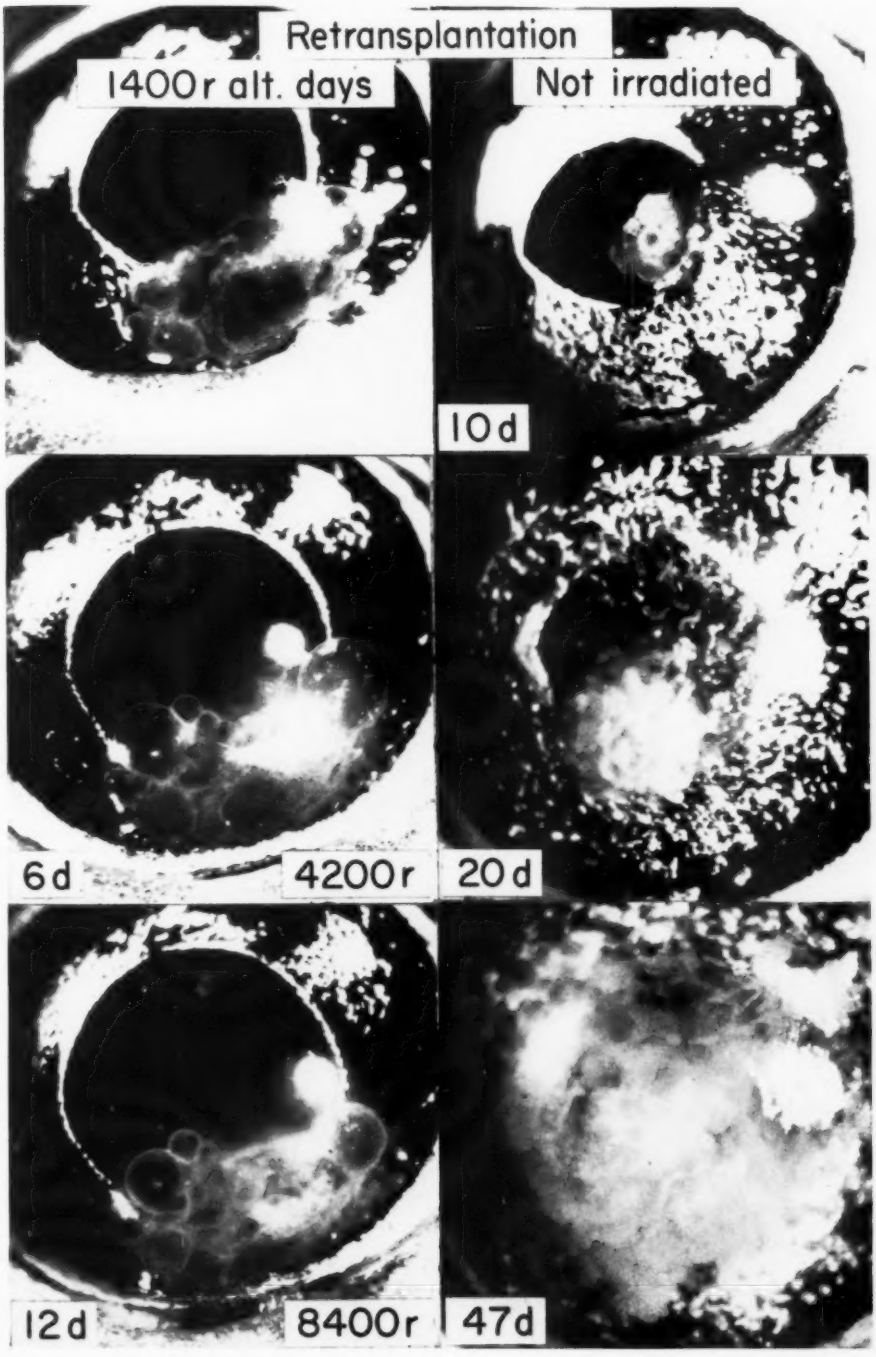

1400r alternate days
21d
11,400r
7d
4200r
30d
12,600r
14d
8400r
49d

PLATE 60

FIGURE 10.—Retransplantation of irradiated intraocular tumors.

The photographs on the right illustrate the growth of a transplant from the tumor shown in figure 2, which had previously received 2,000 r as a single dose. The transplant grew in a normal manner, nearly filling the anterior chamber by the 47th day. By referring to figure 2, it will be noted that the irradiated tumor recovered after a relatively long period of regression. The successful retransplantation, however, does not indicate whether the recurrence is due to recovery of injured tumor cells or to the continued multiplication of cells not affected by the irradiation.

The photographs on the left illustrate the growth of a transplant from a tumor (such as is shown in fig. 6) that had received 1,800 r in fractional doses of 200 r on alternate days. At the time of transplantation no irradiation injury was evident. The upper photograph shows the tumor 20 days after transplantation. Irradiation with 1,400 r on alternate days was then begun and continued until a total of 8,400 r was reached. Growth continued for a time, but after 4,200 r had been given there was little further increase in size, and at 8,400 r there were early signs of regression. This experiment indicates that the previous irradiation had not impaired the capacity of the transplant to grow, nor had it altered its susceptibility to subsequent irradiation. ×9.


Retransplantation
1400r alt. days
Not irradiated
10d
6d
4200r
20d
12d
8400r
47d

STUDIES ON THE INFECTIVITY OF THE MOUSE MAMMARY TUMOR AGENT[1,2]

HOWARD B. ANDERVONT, *National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.*

This paper reports the outcome of four experiments from a series of investigations with the over-all purpose of establishing a basis for quantitative studies on the mammary tumor agent. The aim of these experiments was to establish criteria for determining the minimum infective dose, and the immediate problem was to ascertain whether higher dilutions were capable of infecting mice without producing tumors in them. This possibility deserved attention because it was known that tumors do not appear in all mice known to possess the agent, and studies in this laboratory (*1*, *2*) had shown that the agent was transmitted through three or four successive generations of mice without producing tumors.

In the present experiments, inbred mice, or their hybrids, were given dilutions of the agent and then bred to ascertain the occurrence of mammary tumors in two generations of their descendants. This approach was expected to reveal whether all the infected mice developed tumors and, if not, whether one or two generations of their descendants were necessary to demonstrate infection.

It was found that all infected mice did not develop tumors, but tumors arose in their offspring, especially in the offspring of those receiving higher dilutions. A sufficient number of first-generation offspring developed tumors to indicate that the acquisition of one generation was sufficient to establish the infectivity of a given dose.

MATERIALS AND METHODS

All mice were raised in the laboratory of the author at the National Cancer Institute. They had access to Purina laboratory chow and tap water *ad libitum* and were maintained under similar environmental conditions. Suckling female mice presumably free of the mammary tumor agent were used as recipients of the agent. These consisted of members of a strain C colony (*3*) which shows a mammary tumor incidence of less than 1 percent at an average age of 22 months and members of an agent free line of strain C3H mice (*4*) which shows a tumor incidence of

[1] Received for publication October 19, 1950.

[2] The experiments described in this paper were performed at the National Cancer Institute. The manuscript was prepared at the Roscoe B. Jackson Memorial Laboratory.

922686—51——9

3 percent at an average age of 22 months. Both these strains are known to be susceptible to infection with the agent (*1*). In addition, F_1 hybrids derived from strain I females and agent-free strain C3H males were used. Previous work (*5*) had shown that I × C3H hybrids develop few mammary tumors and are highly susceptible to the agent.

Lactating mice from a strain C colony carrying the strain C3H mammary tumor agent were used as a source of the agent. The ancestors of these animals had been foster nursed by C3H females, and they and their descendants for more than 23 generations have revealed a high incidence of mammary tumors (*1*). Two females that had cast litters on the same day were used in each experiment. They were removed from their litters on the ninth or eleventh day of lactation, kept in isolation overnight (for 16 to 19 hours), killed, and their engorged mammary glands removed. The glands were placed in a tissue mincer and squeezed to extract the milk, which was then strained through a layer of fine gauze. The extracts of lactating mammary glands were pooled, measured, and each cubic centimeter mixed thoroughly with 9 cc. of sterile distilled water. The resultant mixture was centrifuged at 3,000 r. p. m. for 15 minutes, and 0.5 cc. of the supernatant added to 4.5 cc. of distilled water to obtain the first dilution (designated as 10^{-2}) for the inoculation of test animals.

Serial tenfold dilutions were prepared by adding 0.5 cc. to 4.5 cc. of distilled water. A separate sterile 0.5-cc. syringe equipped with a 27-gauge needle was used to prepare each dilution, and the same syringe was used for inoculation of that particular dilution of agent.

All inocula were placed intraperitoneally; each consisted of 0.1 cc. from a 0.5-cc. syringe. The strain and age of the recipient animals are given in the description of individual experiments. When possible, each member of a litter received a different dilution of agent, and each dilution was distributed evenly between younger and older litters. In addition, all members of each litter were inoculated in sequence instead of proceeding from litter to litter to complete the administration of each dilution. This latter procedure was adopted to minimize any deleterious influence that temperature or distilled water may have upon the activity of the agent. These inoculation procedures were accomplished by following a prepared outline based upon the age and number of mice in each litter.

All experiments were carried out at room temperature, and the longest time required was approximately 2 hours between the time the donor animals were killed and the last litter of recipients was inoculated.

Special care was required to prevent leakage of the inoculum from the peritoneal cavity of the suckling mouse. The needle was inserted through the skin of the lower abdomen, passed under the skin for a distance of about 3 mm. and then inserted through the abdominal wall. The point of entrance of the needle through the skin was held between the fingers for 10 to 15 seconds immediately after withdrawal of the needle. The aim of this technique was to produce a seal at the site of needle puncture and to retain the fluid within the peritoneal cavity. Successful inoculation was determined by observing the animal for a short time after the

needle was withdrawn. When leakage occurred the animal was discarded.

All inoculated mice were weaned when 1 month of age, and, when 2 months old, mated to agent-free strain C3H males to avoid the possibility of their offspring acquiring the agent from their male parent (*6*). Such offspring are genetically susceptible to the agent.

Most of the inoculated mice bore one litter; a few, whose first litter died early in life or consisted entirely of males, produced a second litter. When the litters were born, all were reduced to 4 mice (2 females and 2 males) to avoid any possible complications resulting from litter size.

Mice born to the inoculated females were designated first-generation mice. They were weaned when 1 month old and mated to agent-free C3H males 1 month later. Each produced one litter, which was also reduced to 4 mice (2 females and 2 males), and when weaned were designated second-generation mice. Each second-generation female mouse was permitted to bear one litter to an agent-free C3H male. All their offspring were killed within the first 24 hours of life, since the original plan of the experiment was to observe only two generations of descendants from the inoculated mice.

After their litters were weaned, the mice were set aside and examined every other week for the occurrence of mammary tumors. The tumors were fixed in Tellyesniczky's fluid (2 parts formol, 20 parts 70 percent alcohol, and 1 part acetic acid) and stained with hematoxylin and eosin. Microscopic diagnoses were made by Dr. Thelma B. Dunn of the National Cancer Institute, who has described the types of spontaneous mammary tumors found in the mice of this laboratory (*6*).

EXPERIMENTAL PROCEDURES AND RESULTS

In the first experiment, donor mice were removed from their young during the ninth day of lactation, killed 18½ hours later, and an extract of their mammary glands was used to prepare dilutions of the agent. Recipient mice were 9 litters of 10- to 24-day-old agent-free strain C3H mice.

In the second experiment the donor mice were removed from their litters after nursing for 9 days, killed 19 hours later, and dilutions of agent administered to mice of strains C and C3H. Recipient strain C mice belonged to 7 litters and were 7 to 20 days of age; those of strain C3H belonged to 6 litters and were 7 to 26 days old. This experiment was the only one of the series in which different strains of recipient mice were used.

The donor mice of the third and fourth experiments were removed from their young during the eleventh day of lactation. All were killed 17 hours later to prepare dilutions of the agent. In the third experiment 11 litters of 7- to-17-day-old strain C mice were used. In the fourth experiment F_1 hybrid I×C3H females were the recipients. They represented 8 litters and were 7 to 17 days of age.

The results are presented in two tables. The first consists of a summary of the occurrence of mammary tumors in all females, while the second includes only those results that were especially pertinent to the objective of the investigation. In order to present the findings briefly and clearly

922686—51——10

each group of mice that had received a particular dilution of the agent was assigned a number which is found in the first column of both tables. These group numbers are used to facilitate reference to the tables.

The number of inoculated mice in any group was not large, because interest was focused primarily upon the appearance of mammary tumors in their descendants, and, as stated previously, it was thought that during the time required to inoculate a large number of mice the agent may lose its infectivity. This possibility was evaluated by comparing the response of litters that were inoculated first and last in each experiment. The data indicated, that, under the conditions of these experiments, the agent retained its activity.

The first experiment produced results of sufficient significance to justify the inclusion of a number of individuals in table 2.

In table 1 it is seen that 3 mice of group 1 received a 10^{-3} dilution and one died without tumor when 25 months old. In table 2 it is seen that the 3 descendants of this animal (No. 7) developed tumors. Apparently mouse No. 7 was infected but did not develop a tumor.

Both the inoculated and first generation animals of group 2 that died tumor free when 5 months old had tumor-bearing offspring. As shown in table 2, the remaining tumor-free mouse of this group (No. 322 of the second generation) belonged to a family of tumor-bearing animals and, probably, represented an infected individual that did not develop a tumor.

One mouse (No. 24) of group 3 died without tumor when 25 months old. Table 2 reveals that all her offspring developed tumors. Apparently No. 24 was also infected by the 10^{-5} dose.

Group 4 animals of table 1 were of interest. Three mice received the 10^{-6} dilution and died without tumor when 24 months of age. One was assumed to be agent free because she raised 2 first-generation offspring that died without tumors at 24 months of age. The results in this mouse were not clear because neither of her daughters produced second-generation progeny. The remaining mice of group 4 are included in table 2. One inoculated animal (No. 4) had 5 descendants of which only 1 (No. 103) developed a tumor. Mouse No. 103 was considered deficient in the agent because neither of her offspring had tumors, and her tumor was diagnosed as a type B mammary tumor. This type of tumor is known to occur in older mice that are free of the agent (*6*).

The other inoculated mouse (No. 19) of group 4 must have acquired the agent because she had 4 tumor-bearing descendants. Her daughter (No. 85) failed to develop tumor but raised 1 offspring (No. 393) that had a tumor when 11 months old. This was one instance where the agent was propagated through 2 tumor-free mice (Nos. 19 and 85) before producing a tumor in the next generation animal (No. 393).

Group 5 contained an exceptional animal. Data in table 1 show that none of the inoculated mice developed a tumor, but 4 of their 19 descendants did so. In table 2 it is seen that all tumor-bearing mice of the first and second generations descended from 1 (No. 2) inoculated animal. This evidence indicates that mouse No. 2 was infected by the 10^{-7} dilution.

TABLE 1.—*Occurrence of mammary tumors in female mice inoculated intraperitoneally with 0.1 cc. of tenfold dilutions of lactating mammary glands and in two generations of their descendants*

Group No.	Experiment No.	Inoculated mice							First-generation mice					Second-generation mice				
		Strain or derivation	Dilution of agent	Number of mice	Number developed tumor	Average age	Number died without tumor	Average age	Number of mice	Number developed tumor	Average age	Number died without tumor	Average age	Number of mice	Number developed tumor	Average age	Number died without tumor	Average age
						Months		*Months*			*Months*		*Months*			*Months*		*Months*
1	1	C3H	10^{-3}	3	2	12	1	25	3	3	10	0	------	6	6	11	0	------
2	1	C3H	10^{-4}	5	4	13	1	5	5	4	11	1	5	4	3	11	1	21
3	1	C3H	10^{-5}	4	3	13	1	25	5	5	12	0	------	8	8	10	0	------
4	1	C3H	10^{-6}	3	0	------	3	24	6	2	19	4	23	6	3	13	3	17
5	1	C3H	10^{-7}	5	0	------	5	22	8	2	11	6	19	11	2	9	9	21
6	2	C3H	10^{-3}	2	2	10	0	------	2	2	9	0	------	3	3	10	0	------
7	2	C3H	10^{-4}	3	2	9	1	25	4	3	9	1	7	7	7	10	0	------
8	2	C3H	10^{-5}	3	0	------	3	23	3	0	------	3	20	4	0	------	4	18
9	2	C3H	10^{-6}	3	0	------	3	15	3	0	------	3	21	2	0	------	2	18
10	2	C3H	10^{-7}	3	0	------	3	24	5	0	------	5	18	8	0	------	8	20
11	2	C	10^{-3}	4	4	12	0	------	8	8	11	0	------	11	10	11	1	21
12	2	C	10^{-3}	3	1	17	2	20	4	3	8	1	24	7	7	11	0	------
13	2	C	10^{-4}	5	0	------	5	22	10	5	14	5	24	15	5	12	10	20
14	2	C	10^{-5}	5	0	------	5	16	9	0	------	9	23	15	0	------	15	20
15	2	C	10^{-6}	4	0	------	4	24	8	0	------	8	24	13	0	------	13	20
16	3	C	10^{-2}	8	7	13	1	21	10	9	10	1	5	14	12	10	2	20
17	3	C	10^{-3}	7	6	12	1	18	7	5	9	2	15	10	8	11	2	20
18	3	C	10^{-4}	8	7	14	1	23	15	13	11	2	21	26	20	10	6	19
19	3	C	10^{-5}	7	0	------	7	23	11	0	------	11	21	14	0	------	14	20
20	3	C	10^{-6}	8	0	------	8	22	10	0	------	10	24	17	0	------	17	20
21	3	C	10^{-7}	7	0	------	7	19	11	0	------	11	23	21	0	------	21	20
22	4	I×C3H	10^{-3}	6	6	8	0	------	5	5	9	0	------	10	9	10	1	11
23	4	I×C3H	10^{-4}	5	5	11	0	------	4	4	11	0	------	8	8	11	0	------
24	4	I×C3H	10^{-5}	8	1	6	7	24	8	2	10	6	21	13	2	8	11	19
25	4	I×C3H	10^{-6}	6	0	------	6	24	6	0	------	6	24	9	0	------	9	17
26	4	I×C3H	10^{-7}	4	0	------	4	24	4	0	------	4	20	5	0	------	5	18

TABLE 2.—*Results in certain groups listed in table 1 which were pertinent to conclusions derived from the experiments*

Group No.	Experiment No.	Inoculated mice				First-generation mice		Second-generation mice	
		Strain or derivation	Dilution of agent	Mouse number [1]	Age	Mouse number [1]	Age	Mouse number [1]	Age
					Months		*Months*		*Months*
1	1	C3H	10^{-3}	7−	25	81+	8	403+	16
								404+	14
2	1	C3H	10^{-4}	8+	16	94+	11	373+	11
						95+	10	322−	21
3	1	C3H	10^{-5}	24−	25	118+	10	422+	11
								423+	10
								424+	10
4	1	C3H	10^{-6}	4−	23	102−	24	388−	11
						103+	22	390−	20
								392−	20
4	1	C3H	10^{-6}	19−	25	84+	15	396+	12
								397+	6
						85−	22	393+	11
5	1	C3H	10^{-7}	2−	16	66+	9	270+	8
						67+	13	357+	9
12	2	C	10^{-3}	54−	20	41−	24	259+	9
12	2	C	10^{-3}	61−	20	24+	6	235+	10
								236+	10
						25+	9	275+	10
								276+	10
13	2	C	10^{-4}	41−	25	28−	24	307−	20
								308−	20
						29−	24	227−	21
								228−	21
13	2	C	10^{-4}	46−	15	20+	18	262+	11
								263+	12
						21+	13	207−	22
								208+	13
13	2	C	10^{-4}	55−	25	35+	9	246+	10
						36+	16	237+	13
13	2	C	10^{-4}	57−	22	13−	24	241−	20
								242−	20
						14+	16	------	------
13	2	C	10^{-4}	62−	25	26−	24	209−	21
						27−	20	264−	13
								265−	21
16	3	C	10^{-2}	99−	21	150+	9	293+	14
24	4	I×C3H	10^{-5}	131+	6	156+	9	458+	10
								459+	6
						157+	11	------	------

[1] A + sign after the mouse number denotes the occurrence of mammary tumor and a − sign the absence of tumor. The next column shows the age at which the tumor arose or the age at death without tumor.

The results of the first experiment were interpreted as showing that the 10^{-3}, 10^{-4}, and 10^{-5} dilutions of the agent produced infection in all inoculated mice, the 10^{-6} dilution infected 1 of 3, and the 10^{-7} dilution 1 of 5. This was the only experiment in which mice acquired infection from the 10^{-6} and 10^{-7} dilutions.

In the second experiment dilutions of the agent were administered to mice of strains C3H and C. Table 1 data show that the 10^{-3} and 10^{-4} dilutions infected all the C3H recipients. Descendants of the tumor-free animal of group 7 developed tumors. Doses of 10^{-3} and 10^{-4} produced a tumor in only 1 of 8 strain C recipients but the appearance of tumors in

their descendants showed that some of the inoculated mice were infected.

Three mice of group 12 received a 10^{-3} dilution but only 1 developed a tumor. This mouse had 3 descendants, and all had tumors. The remaining inoculated mice and their descendants are included in table 2. One (No. 54) raised 1 daughter (No. 41) that died without a tumor but she, in turn, had an offspring (No. 259) that developed a tumor when 9 months of age. This apparently was another instance where the agent was transferred through 2 generations without evoking a tumor. The other animal (No. 61) of group 12 had 6 descendants, and all developed tumors. Apparently the 10^{-3} dose infected all 3 strain C mice.

Group 13 mice received the 10^{-4} dose, and all are included in table 2 because they gave a clear-cut answer to the primary purpose of the investigation. None of the 5 inoculated mice developed a tumor, but the occurrence of tumors in the descendants of 2 (Nos. 46 and 55) showed that they were infected. Two others (Nos. 41 and 62) were not infected. Offspring of the remaining inoculated animal (No. 57) gave the only equivocal result in the group. Her first-generation daughter (No. 14) developed a tumor at the age of 16 months, but did not raise a litter. The litter mate of No. 14 (No. 13), died tumor-free and her offspring also died without tumor. The evidence was regarded as showing that mouse No. 57 was not infected.

The outcome of the second experiment was interpreted as showing that the 10^{-3} and 10^{-4} doses infected strain C3H and strain C test animals but higher dilutions were noninfective for both strains. Results attending the administration of the 10^{-4} dilution were of special interest here. Response of the inoculated mice indicated that C3H mice only were infected, but the progeny test showed that 2 of 5 C mice were also infected despite the absence of tumors in them.

The third experiment also revealed a sharp end-point of infection at the 10^{-4} dilution. Table 1 data are sufficient to establish this interpretation. Group 16 mice are included in table 2 to show that the tumor-free inoculated mouse (No. 99) had 2 descendants that developed tumors and was, therefore, infected by the 10^{-2} dose. This is the only family history of this experiment listed in table 2, because all first- and second-generation mice of groups 17 and 18 that died tumor-free were descendants from the inoculated mice that failed to develop tumors.

The outcome of the third experiment was interpreted as showing that the 10^{-2} dilution infected all of 8 mice, the 10^{-3} dilution 6 of 7, and the 10^{-4} dilution 7 of 8. None of the animals receiving higher dilutions was infected.

In the fourth experiment, F_1 hybrids derived from strain I females and strain C3H males showed a high degree of susceptibility to the 10^{-3} and 10^{-4} doses, but of 8 mice (group 24) receiving the 10^{-5} dilution only 1 was infected. Table 2 includes this inoculated animal (No. 131) and her descendants, which were the only members of group 24 in which tumors arose.

The results of the experiment showed that the agent was highly active in dilutions of 10^{-3} and 10^{-4}, and the 10^{-5} dose infected 1 of 8 mice. This was the only experiment in which the occurrence of tumors in the inoculated mice provided sufficient evidence to state with certainty which particular animals had been infected.

DISCUSSION

These four pilot experiments do not require extensive discussion because all were designed primarily to test the possibility that administration of infective doses of the mammary tumor agent need not produce tumors in the inoculated animals. In three experiments some inoculated mice remained tumor-free but tumors arose in their descendants, whereas in one experiment the tumor response of the inoculated animals was sufficient to evaluate accurately the number of infected recipients.

Four groups (Nos. 3, 7, 12, 13) of inoculated mice contained 15 individuals that received 10^{-3}, 10^{-4}, or 10^{-5} dilutions and, of these, 6 developed tumors but 9 did not. However, 6 of the 9 tumor-free mice must have acquired the agent because their descendants developed tumors. Hence, the absence of tumors in the inoculated animals was not a reliable criterion of freedom from infection. This suggests that an experiment designed to ascertain the presence of the mammary tumor agent by the inoculation of test animals should include at least one generation of their descendants.

Since these experiments were not designed to carry out final quantitative determinations or to assay the potency of the agent, only small numbers of mice were inoculated with the agent. Furthermore, all interpretations were based upon the outcome in two generations of descendants of the inoculated animals. Additional generations may have produced more findings of interest. This is suggested by the appearance of tumors in some mice of the second generation despite the absence of tumors in their mothers and inoculated grandmothers. It is believed, however, that if larger numbers of mice are used in quantitative or detection studies, one generation of offspring should suffice.

At present the only satisfactory test for the presence of the mouse mammary tumor agent is its ability to produce tumors in susceptible test animals. Furthermore, the establishment of other detection techniques will probably call for the use of test animals. The long latent period between the time a young mouse acquires the agent and the appearance of the resultant tumor is one of the most interesting biologic properties of the agent. But this long latent period, together with the ability of mice to transmit the agent without developing tumors, indicates, that special procedures may be essential for its detection. The experiments reported here show that, following the administration of a small amount of the agent, too much reliance should not be placed upon the occurrence of tumors in the inoculated animals as the sole criterion of infection.

SUMMARY

Serial tenfold dilutions of the mammary tumor agent were administered intraperitoneally to young mice to ascertain whether the tumor response of the inoculated animals could be accepted as an accurate index of infection.

It was found that an appreciable number of the inoculated animals failed to develop tumors but their first- and second-generation descendants did develop tumors. This showed that the agent may infect mice without producing tumors in them.

REFERENCES

(*1*) ANDERVONT, H. B.: Incidence of mammary tumors in mice of strain C3H and in descendants of fostered strain C. J. Nat. Cancer Inst. 10: 193–200 (1949).

(*2*) ———: Studies on the disappearance of the mammary-tumor agent in mice of strains C3H and C. J. Nat. Cancer Inst. 10: 201–214 (1949).

(*3*) ANDERVONT, H. B., and DUNN, T. B.: Efforts to detect a mammary-tumor agent in strain C mice. J. Nat. Cancer Inst. 8: 235–240 (1948).

(*4*) ———: Mammary tumors in mice presumably free of the mammary-tumor agent. J. Nat. Cancer Inst. 8: 227–234 (1948).

(*5*) ANDERVONT, H. B., SHIMKIN, M. B., and BRYAN, W. R.: Technique suitable for quantitative studies on the mammary tumor inciter of mice. J. Nat. Cancer Inst. 3: 309–318 (1942).

(*6*) ANDERVONT, H. B., and DUNN, T. B.: Attempt to detect a mammary tumor-agent in strain C mice by X-radiation. J. Nat. Cancer Inst. 10: 1157–1190 (1950).

VASCULAR REACTIONS OF NORMAL AND MALIGNANT TISSUES *IN VIVO*. III. VASCULAR REACTIONS OF MICE TO FIBROBLASTS TREATED *IN VITRO* WITH METHYLCHOLANTHRENE [1]

GLENN H. ALGIRE, HAROLD W. CHALKLEY, and WILTON R. EARLE, with the assistance of FRANCES Y. LEGALLAIS, HELEN D. PARK, EMMA SHELTON, and EDWARD L. SCHILLING, *National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.*

INTRODUCTION

For a number of years Earle and collaborators (*1–8*) have carried on a study of the action of 20-methylcholanthrene [2] on mouse fibroblasts growing in tissue culture. Fibroblasts obtained from subcutaneous tissue of a young strain C3H mouse (Andervont substrain) were grown in heterologous media consisting of dilute horse serum, chick embryo extract, and chick plasma clot. From this original tissue culture, strains D (untreated), and H, J, L, N, and O were established and exposed for varying intervals of time to the action of MCA. Morphologic, biochemical, and growth studies were carried on *in vitro*, and at approximately yearly intervals the cultures were tested for their capacity to produce tumors by injection into mice of the same strain (C3H) as the starting material.

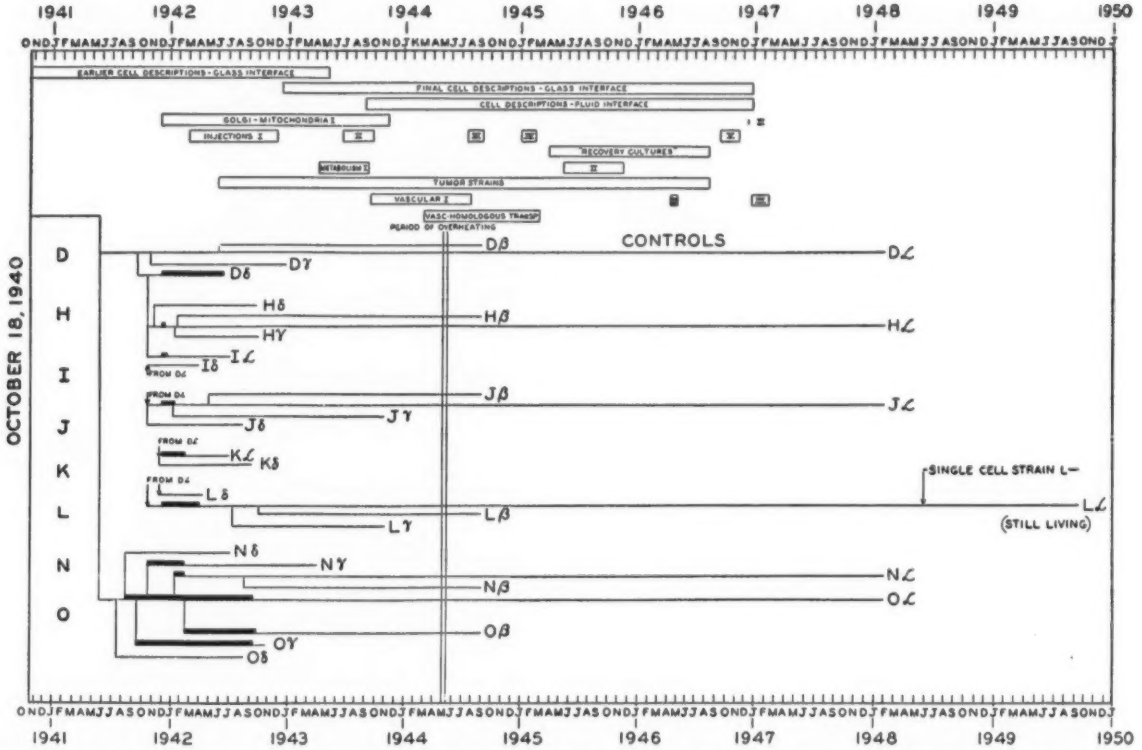
A method for further analysis of the biologic behavior of these cell strains altered *in vitro* and of the tumors obtained on their injection into mice was provided by the development of transparent chamber techniques (*9*, *10*) for the *in vivo* study of the vascular reaction of mice to tissue transplants. By means of these methods, the experiments reported below were carried out to determine what correlations may exist between the ability of these respective cell strains to give rise to sarcoma when injected into mice, and the vascular response of the host to implants of tissue from them.

MATERIALS AND METHODS

All mice used in these experiments were strain C3H (Andervont substrain) males 3 to 6 months old. The *in vivo* method used involves the introduction of a transparent chamber into a skin fold in the mouse. The method of doing this has been described (*10*) and is such that a layer of

[1] Received for publication October 24, 1950.

[2] Hereafter designated as MCA.



TEXT-FIGURE 1.—Chart showing source of each of the strains, the time in MCA, elapsed time after removal from MCA before transplants were made into transparent chamber, and relationship of correlative studies. The time during which each treated culture strain or line was subjected to 20-methylcholanthrene is shown by a very heavy horizontal black line. The bars labeled "injections I–V" indicate the periods of 5 different groups of injections of tissue cultures into mice.

subcutaneous and muscular tissue is made accessible to microscopic observation.

At the time of operation, tissue transplants were made at the site prepared for the chamber. Microscopic study of the vascular reaction of the host was then carried on daily except Sunday. Photographic records, as well as quantitative measurements of the percentage of vascular tissue in both the implant and the surrounding normal subcutaneous tissue layer were used. The method used for measuring the percentage of vascular tissue has been described previously by Chalkley (*11*).

Analysis of the data provided the following information for each implant: 1) The latent period, measured as the interval of time between implantation and first appearance of capillary sprouts; 2) the rate of increase of the vascular bed; 3) the average vascular percentage of vascular tissue after stabilization of the vascular reaction (between the 10th and 16th days following implantation); and 4) the growth rate of the resulting tumors.

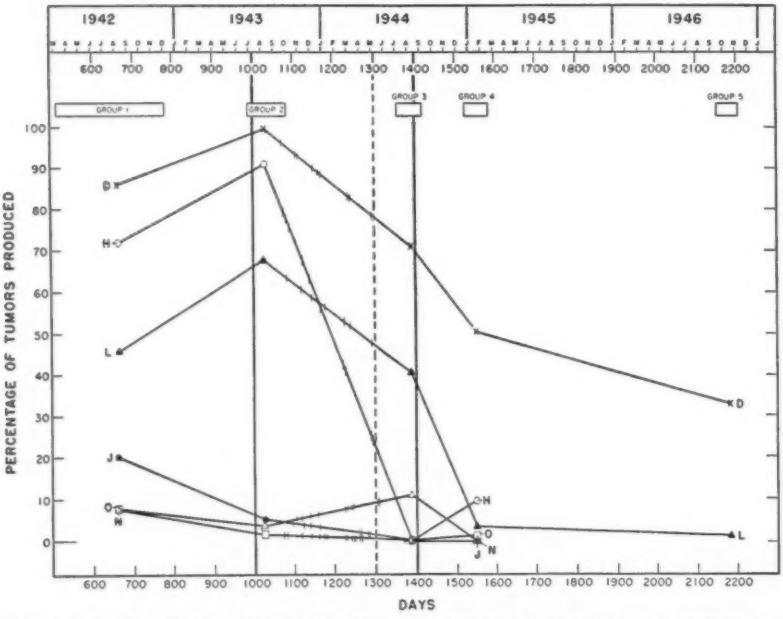
In addition to the quantitative measurements, microscopic observations and photographs provided qualitative information on the reaction of surrounding host capillaries, the time of onset and degree of cellular infiltration within and around the implant, and the development of opacity as an index of tissue damage (*12*).

Some of the mice were saved after removal of the transparent chamber in order to determine whether the implant would continue to grow as a tumor. In other cases, however, the tissues were fixed in Orth's fluid or Zenker-formol, and sections were stained with hematoxylin and eosin, iron hematoxylin, and reticulum stains.

Text-figure 1, from Earle's data, shows the general relation of the experiments reported herein to the design of the entire study, as indicated in the horizontal bars marked vascular I, II, and III. This text-figure shows the source of each of the strains, the time that each strain was exposed to a concentration of 1 gamma of MCA per ml. of culture fluid and the elapsed time after removal from MCA before transplants were made into the transparent chamber. These transplants were made 10 to 15 days after subculture of the cells *in vitro*. From 6 to 10 mice were used for each cell strain studied. Transplants were made of each of the following strains of cells cultivated *in vitro;* D, H, J, L, N, and O. Controls consisted of transplants from normal fibroblasts cultivated *in vitro* (designated F) and of transplants consisting only of plasma clot which had been treated in the same manner as the cell culture.

Twenty-four hours prior to the operation for insertion of the chamber, 2 drops of neutral red solution in balanced saline, containing 5 mg. of dye per ml. were added to each culture flask. At the time of operation the clot supporting the stained cells was cut into rectangular pieces, approximately 4×6 mm. The pieces were shifted to a spot plate and transferred to the tissues of the transparent chamber within from 10 to 20 minutes. Precautions against drying were taken at all times. Figure 1 shows neutral red stained cells contained within a fragment of plasma clot

within the chamber. In this figure the clot containing the cells fills the whole central part of the picture, the cells appearing as a finely granular background within the mass. Text-figure 2 shows the chronological relationship between the transparent chamber studies and previous data of Earle, Shelton, and Schilling (*6*) on tumor percentage following reinjection of the cell strains cultured *in vitro*. Injections of each cell strain were made into groups of mice at intervals, as indicated (text-fig. 2). The major part of the studies reported here are concerned with the vascular reaction to the cell strains cultured *in vitro* during the period between 1,000 and 1,400 days after the explantation of the original tissue culture.



TEXT-FIGURE 2.—Chart showing the change in each cell strain with time, with respect to the number of tumors produced on injection into mice [data of Earle, Shelton, and Schilling (*6*)] and in relation to time of transplantation into transparent chamber. The short vertical bars along the curves indicate time of transplantation into transparent chambers. The two heavy solid vertical lines indicate the period during which the major number of injections were made into transparent chambers. The dotted vertical line indicates the time of overheating of the cultures, as previously described by Earle (see fig. 1).

During this time interval (text-fig. 2) each cell strain except N showed a drop in the percentage of tumors produced. Strain L fibroblasts, carried for a longer period in culture were transferred to transparent chambers at intervals beyond the 1,400-day period indicated above. The criteria described above were used to classify the results into four categories as follows:

1. Tumor growth indicated by translucent progressive growth or diagnosis from histologic sections.

2. Temporary growth and survival for the duration of the preparation, but without progressive growth after removal of the window.

3. Temporary growth followed by regression during the interval of study.

4. Necrosis of the implant.

RESULTS FROM *IN VITRO* CULTURES

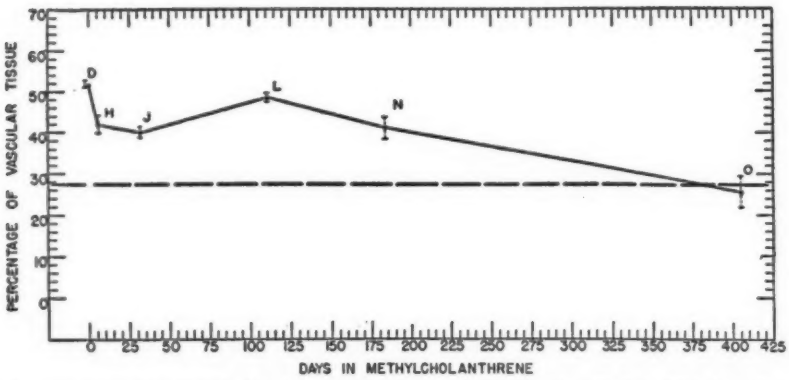
The data summarized here are based on approximately 50 transplants from *in vitro* cultures made into transparent chambers. Six of these grew as tumors, as evidenced by histologic study, or by their continued growth in the mouse after removal of the transparent chamber at the end of the *in vivo* observations. Five of these tumors occurred in the D series, and 1 was the first implant from the H series. Of the transplants which failed to grow continuously as tumors the results varied from temporary growth and survival, or temporary growth followed by regression, to early necrosis (table 1). In the analysis of data, neither the time interval for the appearance of capillary sprouts nor the rate of increase of the vascular bed was significantly different in the various cell strains.

TABLE 1

Cell strains	Days in MCA	Group 2 injection percent "takes"	Average percent vascular tissue 10–15th day	Group 3 injections percent "takes"	Tumor growth	Temporary growth and survival	Temporary growth followed by regression	No growth
D	0	100	52	71	6	0	0	0
H	6	90	42	0	1	2	0	1
J	32	5. 3	40	0	0	0	0	6
L	111	68	48	41	0	6	4	6
N	184	3. 6	40	11. 1	0	0	0	3
O	406	1. 7	30	0	0	0	0	5

STRAIN D

During the period of the transparent chamber studies, data from the paper of Earle, Shelton, and Schilling [(*6*), text-fig. 2], show that strain D (untreated) produced the highest percentage of tumors of any of the *in vitro* strains. At the beginning of the period the percentage of tumors produced was 100; this dropped to 71 percent at the end of the interval. In the transparent chambers new capillary sprouts made their appearance after a latent period of between 5 and 6 days. This capillary proliferation was associated with a strong vascular reaction in the surrounding tissues, with capillary and venous dilatation and irregularity, as shown in figure 2. The average vascular level maintained in the area of the implant during the period of stabilization (10th to 16th day), was 52 percent (text-fig. 3). Typical growth from a strain-D implant at this stage, when it had a vascular percentage of 59, is shown in figure 3. These transplants were translucent, with a minimum of surrounding leukocytic reaction.



TEXT-FIGURE. 3.—Graph showing vascular levels at stabilization (10th to 16th day after inoculation), in relation to average vascular level in tissue surrounding the implant.

That these characteristics of the D strain were associated with their ability to grow as tumors, is borne out by the fact that 4 of the 6 implants continued to grow when the animals were retained after removal of the transparent chamber, while the remainder were diagnosed as tumors on the basis of histologic sections (fig. 4).[3]

STRAIN H

That the H strain cultures (treated with MCA for 6 days) underwent a change in their capacity to produce tumors on subinoculation at some time during the 1,000–1,400-day period included in this experiment, is shown in text-figure 2. This shows that the percentage of tumors produced was 90 percent at the beginning of this period, but 0 percent at the end.

In accord with the results of Earle, is the fact that only the first one of the transplants into the transparent chamber produced a tumor, as shown by its progressive growth and by histologic section. Capillary proliferation occurred after latent periods ranging from 6 to 9 days. Only the first transplant, however, *which eventuated in a tumor*, was translucent and showed dilatation of the capillaries within the tissues surrounding the explant. Figure 5 shows the vascular pattern of this tumor on the 23d day after inoculation. The average vascular percentage was maintained at 42 during the period of stabilization (text-fig. 3).

The remainder of the transplants from strain H cultures failed to produce tumors. Figure 6 shows the typical appearance of the reaction to this group on the 19th day after transplantation, no excessive dilatation of capillaries in the surrounding tissue, no increased translucency of the implant area as compared with the surrounding tissues, and differentiation of the vascular bed into arterioles and venules rather than the large, sinusoidal capillaries characteristic of a progressively growing sarcoma (compare fig. 5).

[3] Acknowledgment is made to Dr. Thelma B. Dunn, Pathology Section, National Cancer Institute, for histologic examination and diagnosis of the sections.

Strain J

Data from the paper of Earle, Shelton, and Schilling [(*6*), text-fig. 2] show that strain J (treated for 32 days with MCA) gave 5.3 percent tumors at the beginning and 0 percent at the end of the period of the transparent chamber studies.

No tumors resulted from the eight implants into the transparent chamber. Capillary proliferation began after a latent period of 5 to 7 days. The implants became more opaque as both leukocytes and a few capillaries entered the area. The general vascular reaction in and around the area of the implant was much much less marked than with either D or H. Figure 7 shows the typical reaction after 8 days, with the arrow indicating the moderate vascular reaction in the implant area. The average vascular percentage was maintained at 40 during the 10th to 16th days (text-fig. 3).

Strain L

During the period of the transparent chamber studies, data from the paper of Earle, Shelton, and Schilling [(*6*), text-fig. 2], show that strain L cultures (treated with MCA 111 days) gave 68 percent tumors on inoculation into mice at the start of the series of transparent chamber experiments undertaken between the period of 1,000 and 1,400 days, but only 41 percent at the end of this interval.

None of the strain L inoculations into transparent chambers gave tumors which grew progressively after removal of the window. The host vascular reaction, however, resembled that of strain D with respect to capillary proliferation and the severe capillary dilatation in the surrounding tissues (fig. 8). This reaction was definitely more striking than with strains H and J. The average vascular level maintained during the period of stabilization (10th to 16th day) was 48 percent (text-fig. 3). As in the case of the strain D implants, the L-strain cell mass remained translucent during the entire observation period of from 12 to 24 days.

Strain L cultures were continued in tissue culture after the other strains were discarded. It was possible, therefore, to reexamine the vascular reaction to this strain in transparent chamber preparations at intervals of approximately 2, 3, and 4 years after the period covered in text-figure 2 (1,000–1,400 days after the original explantation to tissue culture was made). At the end of 2, and of 3 years (approximately 2,000 days from time of original explantation) the vascular reaction to strain L implants was of a less vigorous capillary and venous dilatation around the implant than it had been originally. The results at the end of 4 years, however (approximately 3,000 days from the time of original explantation) were in distinct contrast to those obtained during the periods of from 1,000 to 2,000 days after the original explantation, as described above. Transplanted strain L cells elicited a rich capillary proliferation and remained translucent during a growth period of 8 to 10 days. Shortly after the 10th day, regression began as indicated by increased opacity and decreased thickness of the growth, coincident with decreased vascular per-

centages resulting from narrowing and occlusion of capillaries. The remarkable changes in the behavior of strain L implants after this 4-year interval may be seen by comparison of figures 9 and 10. Figure 9 shows the translucency and large, sinusoidal capillaries typical of a strain L vascular supply on the 16th day after implantation during the initial study (1,000 to 1,400 days after explantation of the original culture). Figure 10 shows, in marked contrast to this, the opacity and narrowed vessels characteristic of the strain L reaction on the 14th day after inoculation 4 years later.

Strain N

Data from the paper of Earle, Shelton, and Schilling [(*6*), text-fig. 2] show that strain N cultures (treated with MCA for 184 days) produced 4 percent of tumors on inoculation into mice near the beginning and 11 percent at the end of the period of the transparent chamber studies. However, since only 55 and 27 mice respectively were used to establish these two points, the rise of strain N from 4 percent to 11 percent is of dubious statistical significance. No tumors resulted from the inoculation of this cell strain into 10 transparent chambers. The implants were characterized by the production of a heavy leukocytic reaction within and around the transplant. The average vascular percentage maintained during the period of stabilization was definitely lower than that of D and L, and averaged 40. Figure 11 shows a strain N vascular reaction on the 11th day, indicating the mild reaction in contrast to strains D and L (figs. 2, 8, and 9).

Strain O

Strain O cultures [treated with MCA for 406 days, (text-fig. 2)] produced the fewest tumors on inoculation into mice of all the *in vitro* strains, with 2 percent at the beginning and none at the end of the interval during which transplants were made into transparent chambers.

These strain O implants were characterized by absence of capillary dilatation in the surrounding tissues. The degree of vascularization, due to capillary proliferation within the implant, was the lowest of any seen for the cell strains, averaging 30 percent during the period of stabilization. Figure 12 shows the opaque appearance of an implant from strain O on the 7th day, indicative of necrosis.

Strain F

Implants were made into transparent chambers from tissue cultures of fibroblasts which had not been treated with MCA (Earle's series 223 and 224). These had been injected into mice repeatedly with no tumor production up until this time, series 223 having had 193 negative injections up to June 1946 when the series was lost, series 224 having had 304 negative injections through June 1947 and thereafter gave sarcomas in a number of instances.

In contrast to strain D, described above, as having undergone 1,100 days of *in vitro* cultivation, series 223 and 224 had been carried *in vitro*

prior to inoculation into transparent chambers for 322 days and 563 days respectively.

All of the four implants made from series 223 developed similarly. There was only moderate leukocytic infiltration. New capillary sprouts were seen in 5 days and attained a level of 45 to 50 percent, which was maintained for a period of from 4 to 26 days and then subsided to the average value for connective tissue. These implants differed from those of strains D and L in their failure to arouse capillary dilatation in the surrounding tissue, and in their general opacity (*cf.* fig. 13, for instance, with figs. 6 and 12). The vascular reaction to these implants, however, was not essentially different from that resulting from implantation of strain H (except the first), J, N, and O. Figure 14 shows, in a stained section, the granulation tissue reaction elicited by such strain F cells. Implants from series 224 elicited less vascular reaction, attaining an average level of 33 percent during the 10- to 16-day period of stabilization.

Plasma Clot

As a further control four implants were made of cell-free plasma clot, which had been treated in all other respects as those which contained cell cultures. These clots were used approximately 2 weeks after being prepared and 24 hours after addition of neutral-red solution to the flask, fluid changes having been carried out routinely.

These implants aroused a slight to moderate leukocytic reaction which subsided after 7 to 10 days. Capillary proliferation was substantially less than for any of the implants containing cells, and capillary dilatation in the surrounding tissues was negligible.

DISCUSSION

The degree of vascular reaction to transplants from tissue cultures (text-fig. 3), was found to run parallel with previous results [Earle and Nettleship (*2*), Earle, Shelton, and Schilling (*6*)] on the capacity of these culture strains (D, H, J, L, N, and O) to give rise to sarcomas. Attention is called, also, to the general similarity of the graph (text-fig. 3) to graphs for data on the average increase in width of cultures (*1*), on the rate of growth of the tumor subinoculations (*8*), the influence of pH upon anaerobic glycolysis (*4*), and of the percentage of cells exhibiting distally located Golgi material (*5*). The similarity of the above indices may be purely coincidental, or may well represent simply various aspects of the growth characteristics of the various cell strains. It seems clear, however, that the ability of the transplanted cell mass to undergo progressive growth as a sarcoma must be directly associated with the capacity for continued endothelial proliferation.

Four general reaction patterns were noted in this series of experiments: progressive growth; temporary growth and survival; temporary growth followed by regression; and no growth. Each strain of cells exhibited characteristic reaction patterns, strains D and L being most distinctly different from the remainder, as may be seen from the ensuing description.

922686—51——11

Those implants of strains D and H which grew progressively as tumors obtained a rich vascular supply of large sinusoidal capillaries. Enlargement of surrounding vessels was a prominent feature. The tumors were translucent in contrast to the surrounding subcutaneous tissue and leukocytes. Implants of strain L from the early series (between 1,000 and 1,400 days after the original explantation) remained translucent during the entire observation period of 12 to 25 days and developed a rich blood supply, with dilatation of surrounding blood vessels, but did not grow progressively after removal of the chamber. The reason for this lack of continued growth is obscure, since implants into mice of entire cultures from Carrel flasks during the time of this study gave rise to between 41 and 68 percent sarcomas (table 1). Besides the factor of the removal of the transparent chamber, a possible explanation for the resorption of the L transplants might be the smaller size of the cell clumps used in these studies as compared with the large inoculum used in previous experiments (*2*).

Subsequent transparent chamber observations of strain L implants made over 4 years later indicated a change toward temporary growth of the graft for 8 to 10 days, followed by the development of opacity and regression. The picture was similar to that of the development of natural resistance encountered when mammary tumors were transferred to the anterior chamber of the eye (*14*) or to the subcutaneous tissue[4] of a resistant mouse strain.

Strain O, the most altered morphologically, which had been treated the longest period of time with carcinogen, gave the least vigorous vascular reaction. This failure of continuing capillary proliferation appears to be associated with the failure of strain O cells to produce sarcomas after injection into mice.

Plasma clot alone aroused less vascular response than any of the cell clumps, indicating that this material produced merely a mild type of foreign-body reaction, and that the presence of cells was necessary for the production of a more vigorous vascular proliferation.

Fibroblast series 223 was lost in tissue culture without having produced sarcomas after 193 injections. Series 224 is anomalous, and renders any interpretation more ambiguous, since at approximately 750 days after these studies it gave rise to sarcomas on injection. The interpretation of this result must await further study.

With increased time in tissue culture after removal of the carcinogen there occurred a decrease in the ability of each cell strain to give rise to sarcomas when injected into strain C3H mice [text-fig. 2 and ref. (*6*)]. Earle, Shelton, and Schilling (*6*) have proposed, as a possible explanation for these results, that the growth of the cultures in an entirely heterologous media for such an extended period of years has gradually altered the characteristics of the cells to where they can less easily live and give rise to sarcomas when injected. The results reported here on the *in vivo* observations, give some experimental support to this view, in regard to the

[4] Unpublished data, G. H. Algire.

ability of the various strains to elicit capillary proliferation. This concept is given further support in comparing the behavior of strain L cells which were transplanted into transparent chambers at intervals over a period of more than 4 years, and during this time underwent a distinct alteration in their reaction with the host. This alteration appeared to be such that the interaction of graft and host tissue resembled that characteristic of the phenomena in natural resistance to tissue transplantation.

Whether MCA, an unknown carcinogen, or the heterologous media is responsible for the initial carcinogenic transformation is beyond the scope of this paper. However, the results presented here suggest that the heterologous media or other factors may so alter the properties of the cells as to mask their neoplastic tendencies. If this hypothesis is correct, further consideration should be given to the rate of the cell change and its influence in masking or modifying the cellular reaction to carcinogens.

SUMMARY

Experiments were carried out to determine what correlations may exist between the neoplastic properties of Earle's *in-vitro*-cultured and MCA-exposed cell strains and the host cellular and vascular reaction to implants of such altered cells made into transparent chambers *in vivo*.

It was found that the vascular reaction elicited by each of the cell strains showed similarities to Earle's data on percentage of tumors produced, average increase in width of cultures, and rate of tumor growth.

Progressive growth was shown by some implants from strains D and H. Temporary growth and survival for at least 20 to 30 days was shown by strain L implants in an early series, but studies made later resulted in temporary growth for 8 to 10 days, followed by regression, such as was found in related studies to be characteristic of natural resistance. Early necrosis and foreign-body reaction were characteristic of strains J, N, and O.

These results are discussed in relation to the possibility that growth *in vitro* for a prolonged period of time in heterologous media may so alter the characteristics of the cells as to mask their carcinogenic potencies.

REFERENCES

(*1*) Earle, W. R.: Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. J. Nat. Cancer Inst. 4: 165–212 (1943).

(*2*) Earle, W. R., and Nettleship, A.: Production of malignancy in vitro. V. Results of injection of cultures into mice. J. Nat. Cancer Inst. 4: 213–227 (1943).

(*3*) Nettleship, A., and Earle, W. R.: Production of malignancy in vitro. VI. Pathology of tumors produced. J. Nat. Cancer Inst. 4: 229–248 (1943).

(*4*) Burk, D., Earle, W. R., and Winzler, R. J.: Production of malignancy in vitro. VII. Metabolism and biotin vitamer content. J. Nat. Cancer Inst. 4: 363–372 (1944).

(*5*) Dalton, A. J. and Earle, W. R.: Production of malignancy in vitro. VIII. Observations on the mitochondria and Golgi material. J. Nat. Cancer Inst. 4: 539–554 (1944).

(6) EARLE, W. R., SHELTON, E., and SCHILLING, E. L.: Production of malignancy in vitro. XI. Further results from reinjection of in vitro cell strains into C3H mice. J. Nat. Cancer Inst. 10: 1105–1113 (1950).

(7) SHELTON, E., and EARLE, W. R.: Production of malignancy in vitro. XII. Behavior of recovery cultures. J. Nat. Cancer Inst. (in press).

(8) EARLE, W. R.: A summary of certain data on the production of malignancy in vitro. A. A. A. S. Res. Conf. on Cancer 139–153 (1945).

(9) ALGIRE, G. H.: An adaptation of the transparent chamber technique to the mouse. J. Nat. Cancer Inst. 4: 1–11 (1943).

(10) ALGIRE, G. H., and LEGALLAIS, F. Y.: Recent developments in the transparent chamber technique as adapted to the mouse. J. Nat. Cancer Inst. 10: 225–253 (1949).

(11) CHALKLEY, H. W.: Method for the quantitative morphologic analysis of tissue. J. Nat. Cancer Inst. 4: 47–53 (1943).

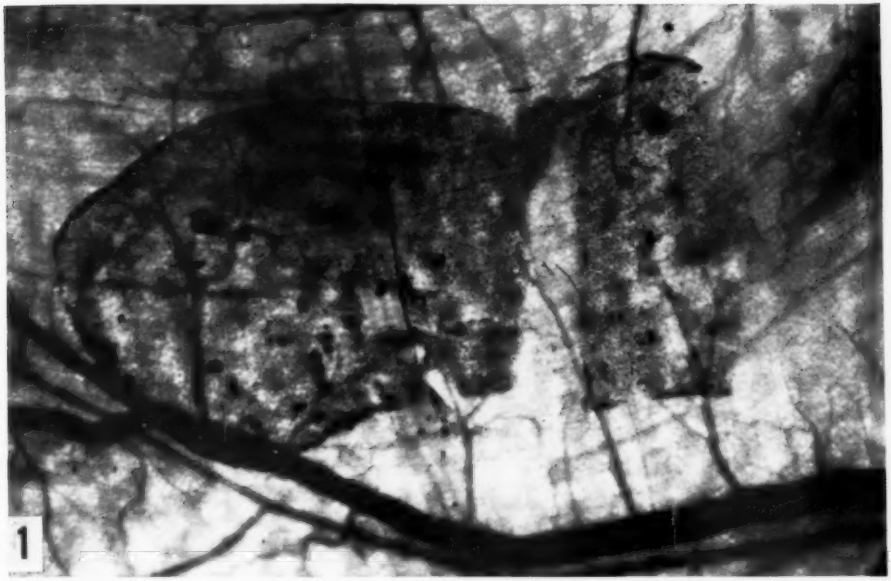
(12) YOUNGNER, J. S. and ALGIRE, G. H.: The effect of vascular occlusion on transplanted tumors. J. Nat. Cancer Inst. 10: 565–580 (1949).

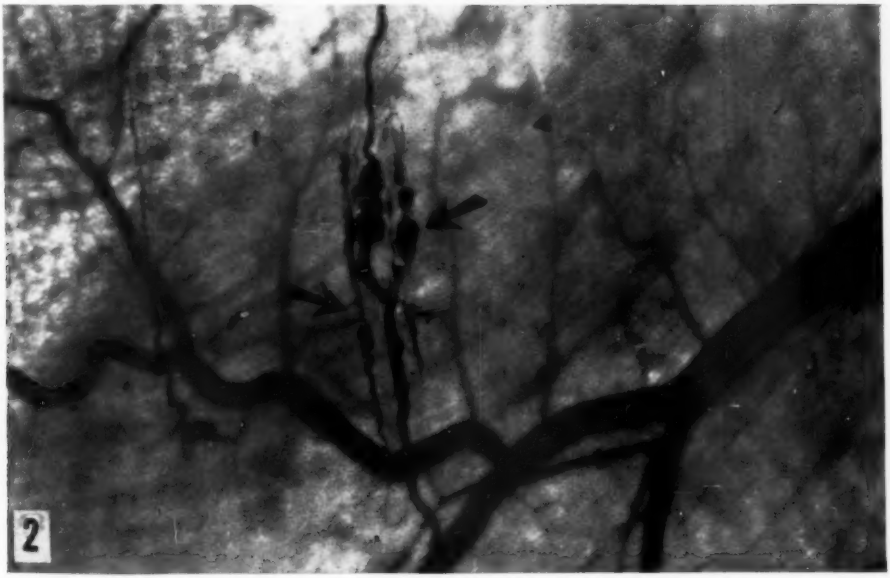
(13) ALGIRE, G. H. and CHALKLEY, H. W.: Vascular reactions of normal and malignant tissues in vivo. I. Vascular reaction of mice to wounds and to normal and neoplastic transplants. J. Nat. Cancer Inst. 6: 73–85 (1945).

(14) BROWNING, H. C.: Heterologous and homologous growth of transplants during the course of development of spontaneous mammary tumors in C3H mice. J. Nat. Cancer Inst. 8: 173–190 (1948).

PLATE 61

FIGURE 1.—Plasma clot containing neutral-red stained cells within the chamber. × 20.
FIGURE 2.—Venous tortuosity and capillary growth (arrows) 7 days after inoculation of strain D cells. × 20.





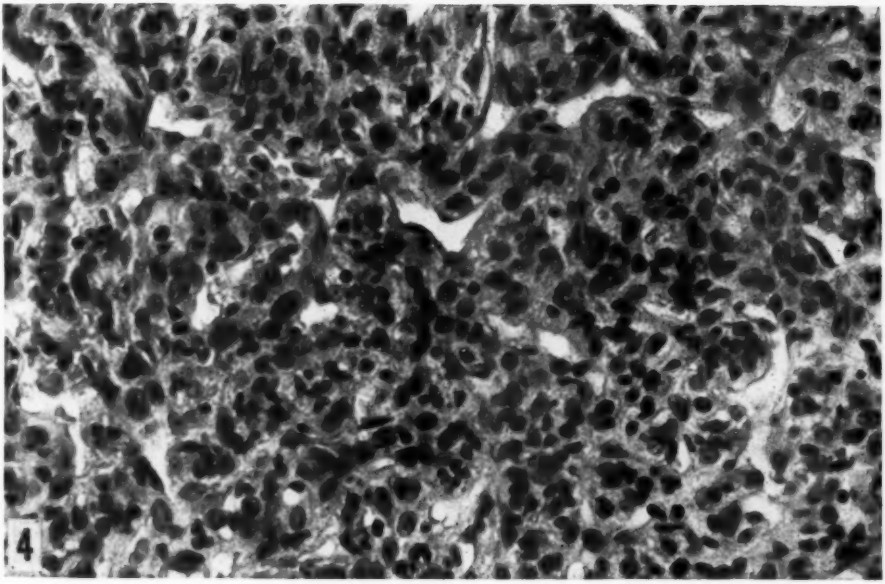
Algire *et al.*

922686—51——12

PLATE 62

FIGURE 3.—Vascular pattern in tumor arising from strain D cells, 13 days after inoculation. × 50.

FIGURE 4.—Sarcoma from *in vitro*, cultured cells of strain D. Zenker-formol fixation hematoxylin-eosin stain. × 500.

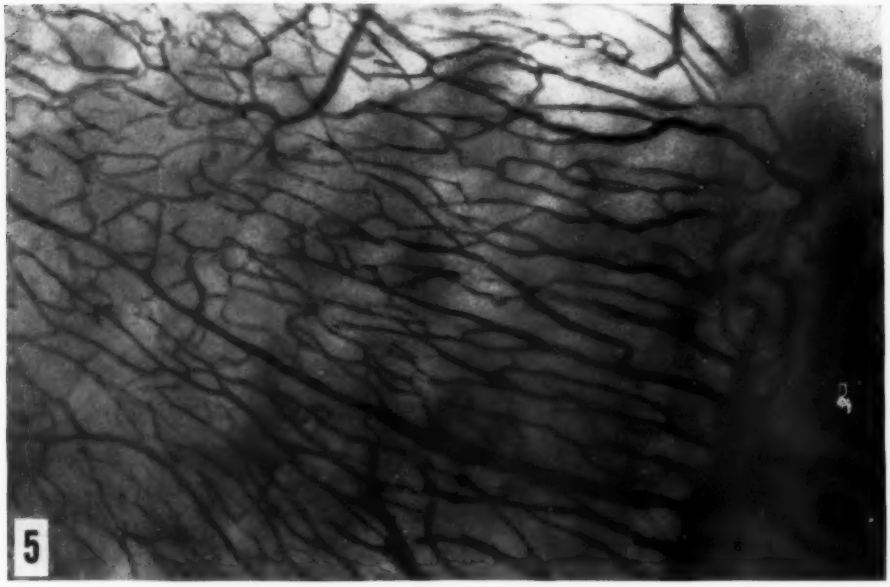


Algire *et al.*

PLATE 63

FIGURE 5.—Vascular pattern in tumor arising from strain H cells, 23d day after inoculation. × 50.

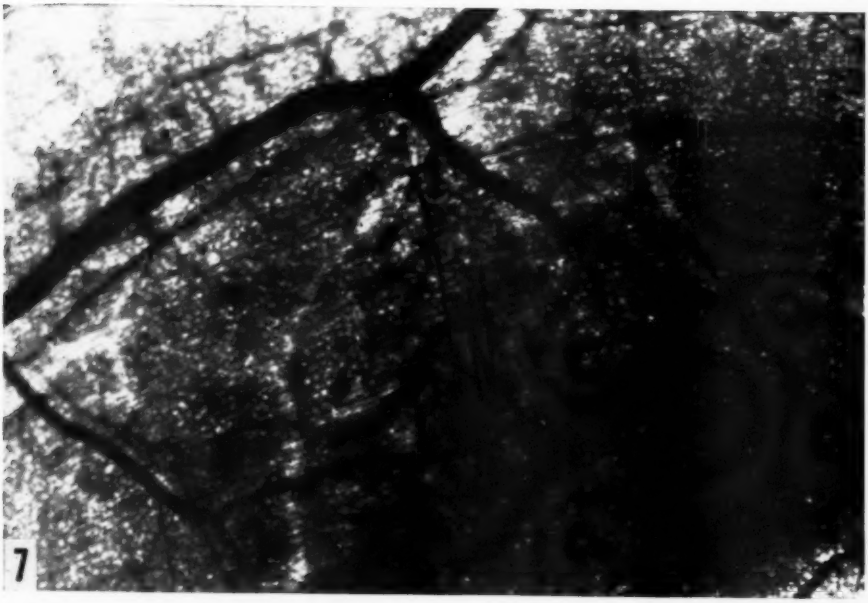
FIGURE 6.—Vascular reaction to strain H cells, 19th day after inoculation. × 50.



Algire *et al.*

PLATE 64

FIGURE 7.—Mild vascular reaction to strain J cells, 8th day after inoculation. × 25.
FIGURE 8.—Vascular reaction to implant of strain L cells, 23 days after inoculation. × 50.

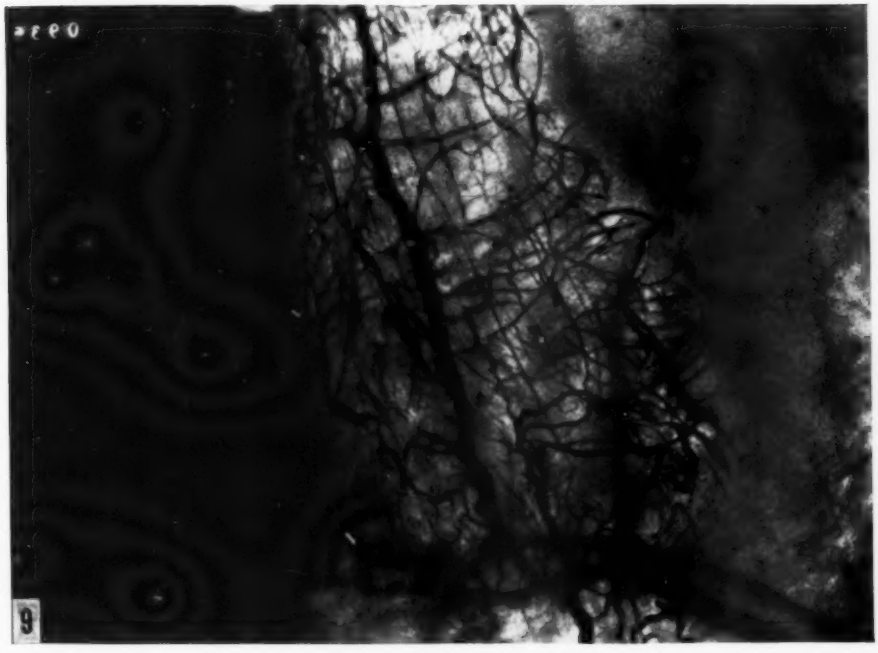


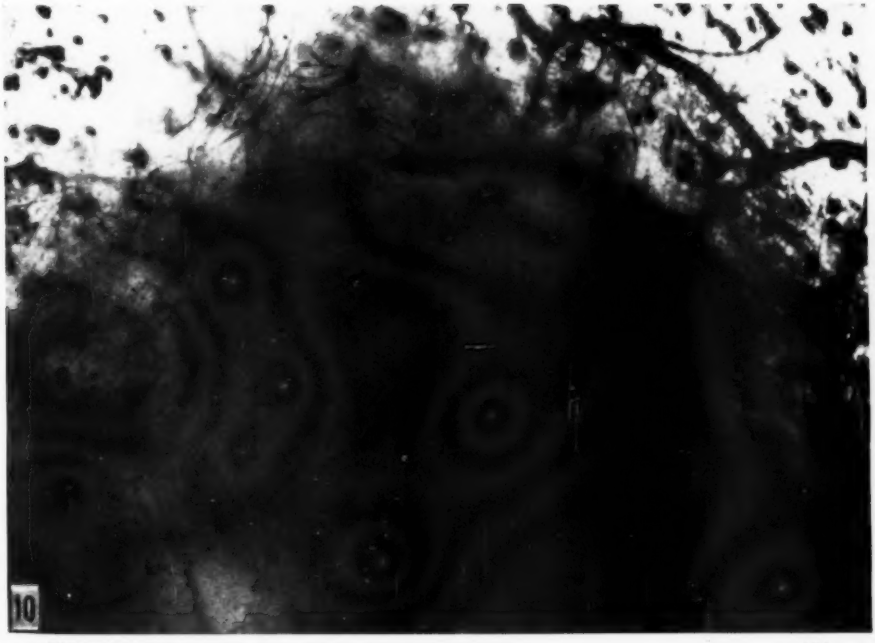
Algire *et al.*

PLATE 65

FIGURE 9.—Translucent implant from strain L, with rich vascular supply typical of reaction in 1943—16th day after inoculation. × 50.

FIGURE 10.—Opacity of implant from strain L, after initial temporary growth, typical reaction in 1948—14th day after inoculation. × 30.





922686—51——13

PLATE 66

FIGURE 11.—Moderate vascular reaction of strain N cells, 11th day after inoculation. × 20.

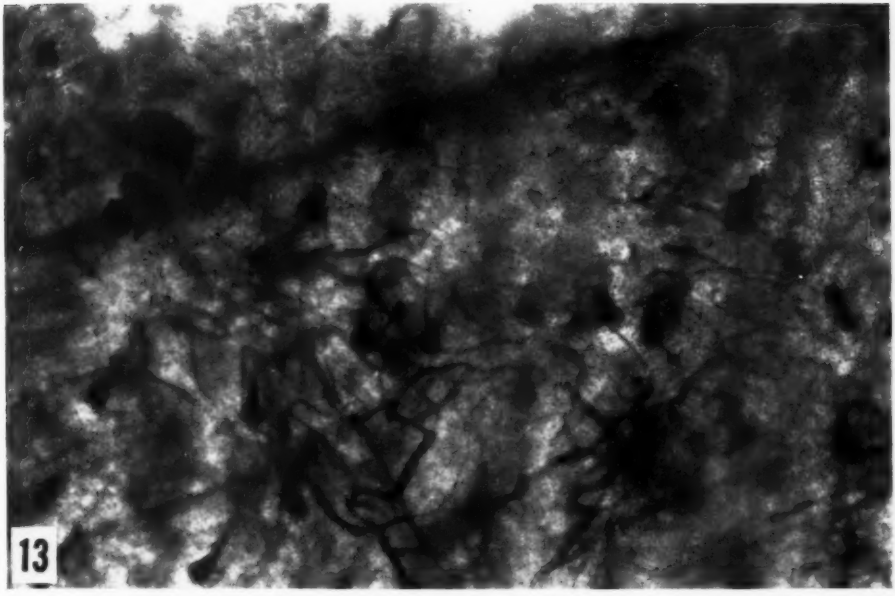
FIGURE 12.—Opacity of implant from strain O, 7 days after inoculation. × 10.

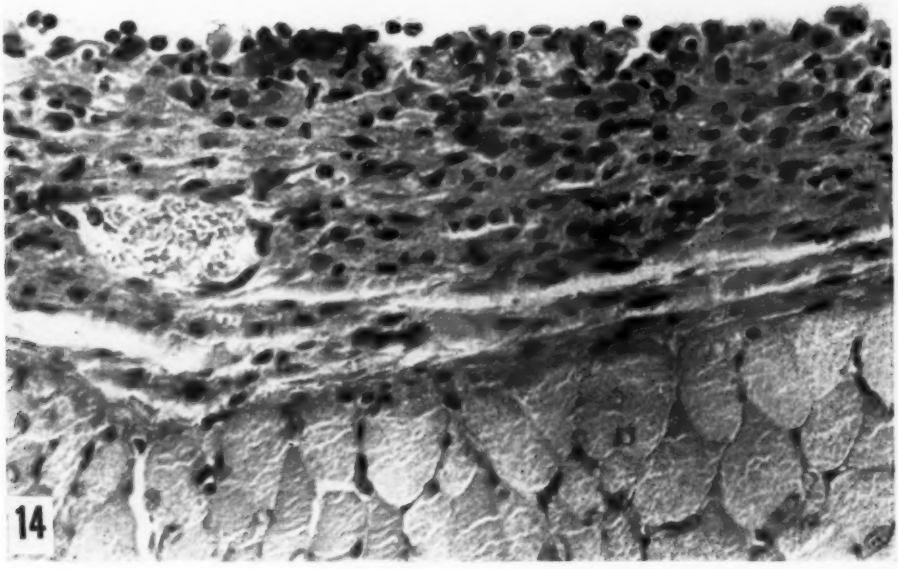
Algire *et al.*

PLATE 67

FIGURE 13.—Vascular reaction to fibroblast culture showing absence of increased translucency in implant area. 12th day.

FIGURE 14.—Granulation-tissue reaction to strain F cells. Zenker-formol fixation, hematoxylin-eosin stain. × 500.





Algire *et al.*

922686—51——14

STUDIES ON THE OCCURRENCE OF SPONTANEOUS HEPATOMAS IN MICE OF STRAINS C3H AND CBA[1,2]

HOWARD B. ANDERVONT, *National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.*

Although primary tumors of the liver have been observed in stock (*1*, *2*) and various strains of inbred mice, this paper refers only to investigations with strains C3H and CBA because, to date, they are regarded as the most susceptible of the inbred strains and have received the most consideration. Both strains were established by Strong (*3*, *4*), who has called attention to their common ancestry (*5*). During their development selection toward susceptibility to the occurrence of hepatomas was not practiced, and their genetic predisposition to this type of tumor was revealed by investigators who autopsied the older animals.

The tendency of strain CBA to develop spontaneous hepatomas was noted in this country by Strong and his colleagues and in England by Gorer, as well as by Pybus and Miller. Strong (*4*) autopsied 71 females and found the lesion in 2 mice, 1 of which was 581 and the other 602 days of age. Strong and Smith (*6*) reported that 6 males and 8 females had developed hepatomas at an average age of 20.4 and 22 months, respectively. Later Strong, Smith, and Gardner (*7*) observed the presence of spontaneous hepatomas in 42 CBA mice at an average age of 613 days and, of interest here, stated that the tumors arose in both sexes at approximately equal rates. This represents the only claim, thus far, for an equal sex distribution of this tumor.

Strong published 2 reports in which strain CBA females were used in dietary studies. In the first (*8*), 1 of 63 had a hepatoma; and in the second (*9*), 6 of 81 mice had similar tumors at a mean age of 695 days.

Gorer kept strain CBA mice to ascertain their incidence of spontaneous tumors and noted a sex difference in their susceptibility to the development of spontaneous hepatomas. In one communication (*10*) he reported that of 39 mice of each sex that were over 14 months of age, 14 males and 4 females had developed hepatomas. In another (*11*), he again emphasized the higher susceptibility of males and recorded a case with metastasis to the lungs.

[1] Received for publication October 26, 1950.

[2] The experiments described herein were performed at the National Cancer Institute. The manuscript was prepared at the Roscoe B. Jackson Memorial Laboratory.

922686—51——15

Pybus and Miller (*12*) carried out a thorough study of the incidence of spontaneous tumors in strain CBA by permitting 514 mice to live their allotted spans of life. Of 285 males, 116, or 40.7 percent, had hepatomas at an average age of 28.6 months: the ages of the 116 males ranged between 19 and 37 months. Of 229 females, 61, or 26.6 percent, had the same type of tumor at an average age of 30.1 months: the ages of the tumor-bearing females were between 13 and 45 months. Their findings established the preponderance of hepatomas in males. In a subsequent paper, Miller and Pybus (*13*) again demonstrated a sex difference in susceptibility of CBA mice and found that in hybrids obtained from strain CBA, spontaneous hepatomas occurred more frequently in the males.

The susceptibility of strain C3H mice to spontaneous hepatomas has been noted by several investigators. One report (*14*) from this laboratory recorded the incidence in 165 mice that were over 1 year of age. Eighty-five females showed an incidence of 11.7 percent at an average age of 16.9 months, whereas 80 males showed an indicence of 22.5 percent at a mean age of 15.1 months. Another report (*15*) included 461 strain C3H mice that were over 1 year of age; 141 females showed an incidence of 9.9 percent at an average age of 17.5 months and 320 males an incidence of 26.8 percent at an average age of 15.3 months. Thus, strain C3H males exhibited a higher incidence of spontaneous hepatomas than did the females.

Burns and Schenken also found a higher incidence of hepatomas in C3H males. In one communication (*16*), of 38 untreated breeding males, 16, or 42 percent, had hepatomas at an average age of 15.3 months, and of 46 similar females who lived to a mean age of 12.9 months none had a hepatoma. In a second paper (*17*), they compared the incidence in breeding and virgin C3H mice and found that of 60 breeding males 16, or 27 percent, had hepatomas at a mean age of 15.5 months, whereas of 47 breeding females killed at an average age of 14 months none had a neoplasm of this type.

This brief summary of reports from different laboratories is sufficient to establish that in mice of strains C3H and CBA spontaneous hepatomas are found in the older animals and occur more frequently in the males.

Investigations reported herein were designed 1) to obtain further information on the incidence of spontaneous hepatomas in mice of strains C3H and CBA, 2) to ascertain whether the mammary tumor agent exerted any influence upon the occurrence of the lesion, and 3) to explore the effects of breeding and castration upon the development of hepatomas. The first objective was undertaken because of the divergent results in sex susceptibility of CBA mice reported from England and this country. The second objective stemmed from the availability of a line of agent-free strain C3H mice which presented an opportunity to compare the hepatoma incidence in males, with and without the agent, and to compare the incidence in older agent-free breeding females to the incidence in older males. The latter study was impractical with agent-containing C3H females because virtually all develop mammary tumors at an early age. The

third objective was included because Burns and Schenken (*17*) had suggested that breeding influenced the development of spontaneous hepatomas in strain C3H, and previous investigations in this laboratory (*18*) had shown that gonadectomy of male strain C mice had a pronounced influence upon their susceptibility to induced hepatomas.

EXPERIMENTAL PROCEDURES AND RESULTS

All mice were raised in this laboratory. They had access to an unlimited supply of Purina dog chow or laboratory chow and tap water. The diet was never changed during the course of an experiment. They were kept eight to a cage, housed in wooden boxes 28 cm. long, 18 cm. wide, and 15 cm. deep, and supplied with bedding of sawdust or wood shavings. Some were housed in plastic cages 28 cm. long, 18 cm. wide, and 12 cm. deep.

When males were set aside for observation, litter mates were kept in the same cage to obviate any differences in environment. This procedure is feasible with males of strains C3H and CBA because the breeders show little inclination to fight when removed from females and placed with other males. All males and some females were killed when 15 months old to obtain autopsies of comparable groups at the same age level and to avoid loss of experimental data through post-mortem decomposition.

The presence of hepatomas was usually determined by gross examination, since the lesions in the mouse have a characteristic appearance. They are usually elevated, clearly defined, round or oval masses on the surface or margins of the lobes: some occur as pedunculated masses. They may be yellow or the same color as the liver. Questionable lesions, approximately 5 mm. in diameter, were confirmed by histologic examination. Lesions up to 5 mm. in diameter were disregarded to assure the tabulation of hepatomas only, and to avoid the trouble of examining all minute liver lesions of doubtful significance. The hepatomas included herein were similar in all respects to those described grossly and microscopically by various pathologists (*6*, *11*, *16*, *19*, *20*).

The occurrence of spontaneous hepatomas in 7 series of mice is summarized in table 1. Series 1 consisted of strain C3H breeding females belonging to a line of agent-free mice established in this laboratory (*21*) during 1942. The table includes those of the first 15 generations who survived beyond the age of 15 months.[3]

Mice of Series 2 were also agent-free C3H breeding females derived from the agent-free line but not included in the colony records. They represented 3 experiments in which the animals were born during 1943, 1944, and 1945.

[3] An earlier paper (*21*) presented the incidence of mammary tumors in the first 10 completed generations of this line. Of 276 mice, 9, or 3 percent, developed tumors at an average age of 22 months. The average age of those dying without tumor ranged between 20 and 23 months in different generations.

The results in 5 more generations are presented. Of 151 mice, 9, or 6 percent, developed tumors at a mean age of 21.5 months. The average age at death of the 142 tumor-free mice was 20 months.

Thus, in the first 15 generations of this agent-free line of strain C3H breeding females the mammary tumor incidence was 4.2 percent at a mean age of 21.8 months.

TABLE 1.—*Occurrence of spontaneous hepatomas in mice of strains C3H and CBA*

Series No.	Strain	Sex	Breeders, virgins, or castrates	Mammary tumor agent	Number of mice	Mice with hepatoma / Mice without hepatoma — Age in months															Totals	Incidence
						15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
																						Percent
1	C3H	F	Breeders	Absent	362	1/3	0/5	0/8	2/23	2/15	2/16	1/23	0/18	3/29	28/167	3/13						42/320	11
2	C3H	F	do	do	179		0/4	0/7	0/9	0/4	0/12	0/11	1/7	1/16	1/21	3/32	0/15	1/20	1/6	1/4	0/2	9/170	5
3A	C3H	M	do	Present	40	9/31																9/31	23
3B	C3H	M	Virgins	do	43	5/38																5/38	12
3C	C3H	M	Castrates	do	36	0/36																0/36	0
4	C3H	M	Virgins	do	86	47/39																47/39	55
5A	C3H	M	Breeders	do	60	18/42																18/42	30
5B	C3H	M	Virgins	do	118	32/86																32/86	27
5C	C3H	M	Castrates	do	73	13/60																13/60	18
5D	C3H	M	Breeders	Absent	31	14/17																14/17	45
5E	C3H	M	Virgins	do	48	19/29																19/29	40
5F	C3H	M	Castrates	do	34	2/32																2/32	6
6A	C3H	M	do	do	21	3/18																3/18	14
6B	C3H	M	do	Present	19	3/16																3/16	16
7A	CBA	M	Breeders	do	12	3/9																3/9	25
7B	CBA	M	Virgins	do	68	20/48																20/48	29
7C	CBA	M	Castrates	do	40	8/32																8/32	20
7D	CBA	F	Virgins	do	63	3/60																3/60	5
7E	CBA	F	Breeders	do	29	1/8	0/9	0/2	0/4	0/2	0/1	0/1	0/1									1/28	3

Series 3 was comprised of males from the C3H colony (*22*) that were born during 1942 and 1943. They were litter mates divided between 3 approximately equal groups of breeders, virgins, and castrates.

Series 4 consisted of C3H males derived from a litter sent to this laboratory by another investigator who in turn had procured a litter of C3H mice from this laboratory 5 years previously. Descendants from the litter were kept to ascertain whether a sojourn in another laboratory had altered their incidence of spontaneous tumors. Series 4 mice were born during 1944.

Series 5 represented another experiment in which litter mate C3H males were kept as breeders, virgins, and castrates to compare their hepatoma incidence. They were born between 1944 and 1947 and were derived from the colony or the agent-free line. The experiment was designed to reveal any influence the mammary tumor agent may exert upon the occurrence of hepatomas.

Series 6 males were part of a pilot investigation to ascertain the effects of sex hormones upon the occurrence of spontaneous hepatomas in castrate males. They were castrate controls derived from the C3H colony or the agent-free line and were born during 1947.

Series 7 was comprised of strain CBA mice born during 1947 and 1948. These descended from a litter kindly supplied by Dr. L. C. Strong.

Data in table 1 are interpreted as follows:

1. Confirmation of the occurrence of spontaneous hepatomas in mice of strains C3H and CBA.

2. Confirmation of the greater susceptibility of males. Of 426 intact C3H males, 144, or 33.8 percent, had hepatomas when 15 months old, whereas of 541 intact females, 51, or 9.4 percent, had this type of tumor when over 15 months of age. Of 80 intact CBA males, 23, or 28.7 percent, had hepatomas when 15 months old, and of 92 intact females, 4, or 4.3 percent, had similar tumors when over 15 months of age.

3. Presence of the mammary tumor agent was not essential for the development of hepatomas in C3H mice.

4. Breeding did not alter appreciably the incidence in 15-month-old C3H males. A total of 131 breeders showed an incidence of 31.3 percent; and 295 virgins, an incidence of 34.9 percent.

5. There was considerable variation between the incidence of tumors in different groups of 15-month-old C3H males. Breeders showed incidences of 23, 30, and 45 percent, virgins 12, 27, 40, and 55 percent, and castrates 0, 6, 14, 16, and 18 percent.

6. Castrate C3H males had consistently lower incidences than did their intact litter mates. Of 183 15-month-old castrates, 21, or 11.4 percent, had hepatomas. This incidence is to be compared to that of 33.8 percent in 426 intact males, many of which were litter mates of the castrates. However, in one experiment with CBA males (Series 7) castration did not exert such a decided influence.

Table 2 summarizes the occurrence of hepatomas in castrate males according to the ages at which the mice were gonadectomized. The

TABLE 2.—*Occurrence of spontaneous hepatomas in castrated males of strains C3H and CBA*

Series No	Strain	Mammary tumor agent	Number of mice	Mice with hepatoma / Mice without hepatoma — Age at castration in days								
				31–40	41–50	51–60	61–70	71–80	81–90	91–100	111–120	121–130	151–160
3C	C3H	Present	36	0/12	0/16	0/6	0/2						
5C	C3H	do	73	0/17	3/15	7/10	1/9	0/4	0/1	2/2			0/2
5F	C3H	Absent	34	1/10	1/3	0/7	0/5	0/5	0/1		0/1		
6A	C3H	do	21	0/6	2/3	0/3	1/6						
6B	C3H	Present	19	1/4	1/4	0/3	1/2		0/2		0/1		
7C	CBA	do	40	1/2	1/8	2/13	2/6	0/1				1/0	1/2
Total			223	3/51	8/49	9/42	5/30	0/10	0/4	2/2	0/2	1/0	1/4

only suggestive result was the low incidence in those castrated when 31 to 40 days old. Further studies are necessary to establish a correlation between the age at castration and the incidence of spontaneous hepatomas.

DISCUSSION

The occurrence of spontaneous hepatomas in related strains C3H and CBA needs no discussion, since both strains are known to be susceptible to this neoplasm. The greater susceptibility of males of both strains is also in accord with most earlier findings and shows that the CBA mice maintained in this country and in England do not differ in this respect.

Establishment of a line of C3H mice that were free of the mammary tumor agent permitted a more thorough study of the occurrence of hepatomas in the females. Despite a longer average life span the females showed a much lower incidence than did 15-month-old males of the agent-free line. This emphasized their sex difference in susceptibility and confirmed existing knowledge of the specificity of the mammary tumor agent.

Variations between the incidences of hepatomas in different series of intact 15-month-old C3H males deserve some comment. In these studies the extreme incidences were 12 percent in a group of 43 virgins (Series 3B) and 55 percent in another group of 86 virgins (Series 4). Such differences may be expected when the over-all incidence in 15-month-old males is approximately 30 percent, and should receive serious consideration before conclusions are based upon small numbers of experimental animals.

Fluctuations in the incidence of spontaneous hepatomas between groups of C3H males have been observed repeatedly in this laboratory, and all attempts to determine whether the tumors were more frequent in certain litters or families within the strain have emphasized their random distribution. Hence, the necessity of using either large numbers of animals or conducting repeat experiments in studies on this type of tumor. The use of small numbers of animals is not justified, even when litter mates serve as controls.

In the present investigations breeding did not alter appreciably the development of hepatomas in C3H males: Burns and Schenken (*17*) reported an incidence of 27 percent in 60 breeding C3H males, in contrast to an incidence of 6 percent in 16 virgins, and an incidence of 0 percent in 47 breeding females, in contrast to an incidence of 10 percent in 10 virgins. They commented upon these findings as showing that breeding "favorably influenced the development of tumors in the males and unfavorably influenced the development of tumors in the females." They also stated that "the small number of animals in some groups makes confirmatory experiments desirable." The present findings did not confirm the tentative conclusion of these investigators.

A pronounced sex difference in susceptibility to spontaneous hepatomas has suggested to various investigators that hormonal stimulation may have some bearing upon their development. Miller and Pybus (*24*) gave weekly subcutaneous injections of 0.05 cc. of a 0.06-percent solution of estrone in olive oil to castrate and intact CBA mice of both sexes. There

was an increased incidence of hepatomas in intact males, but a decreased incidence in castrate males as well as in both intact and castrate females. They stated the results were "difficult to assess . . . on account of the earlier death in all classes compared with controls."

Burns and Schenken (*16*) administered from 400 to 3,000 rat units of progynon-B (estradiol benzoate in a solution of sesame oil) to 18 virgin C3H males and found that 10, or 55.5 percent, had hepatomas at an average age of 14.2 months. This incidence was similar to that of 53.3 percent in 30 control animals, but the tumors in the treated mice were found on an average of 1 month earlier than those in the controls. While discussing their results they stated there was "a suggestion that the incidence of hepatomas was higher among the animals which received 3,000 rat units of estrogen." In 15 virgin females that had received subcutaneous implants of either the hypophysis and thyroid gland or the adrenal glands and pancreas, they found 3, or 20 percent, with hepatoma at a mean age of 14.4 months. This incidence was higher than a 0-percent incidence in 24 control females, but since the controls had borne litters the investigators stated "it is not yet possible to conclude that the presence of the transplants increased the incidence of liver tumors . . ."

Schenken and Burns (*23*) also administered testosterone propionate and estrogens to virgin mice to test their effects on the tumor rate. Testosterone propionate was given to 24 males and 24 females which were 10 to 21 days old when the injections were begun. None of the males and only 1 female developed hepatoma, although the average age at death for males was 14.9 months and for females 15.1 months. Ten- to 14-day-old males and females received subcutaneous injections of *a*-estradiol benzoate in a solution of sesame oil. Of 59 males, 15, or 25 percent, had hepatomas at an average age of 15.8 months, and, of 10 females, none had a hepatoma when killed at the same average age. Subcutaneous injections of ketohydroxyestrin dissolved in sesame oil were started when 30 males and 9 females were 10 days to 6 months old. Five, or 17 percent, of the males had hepatomas at a mean age of 19.1 months, but when the females were examined at an average age of 13.5 months, none showed a hepatoma. The authors did not evaluate the results of androgen treatment, and interpreted their findings as suggesting that estrogens elevated the incidence in males and depressed the incidence in females. They stated "the evaluation of the effect of estrogen treatment on the incidence of liver tumors must be considered as tentative because the number of mice in many groups was small."

Shimkin and Wyman (*25*) implanted estrogen-cholesterol pellets subcutaneously into male C3H mice to study primarily the relationship between the dose of estrogen and the mammary-tumor response. Pellets containing different concentrations of diethylstilbestrol, estradiol, or triphenylethylene were implanted into 486 1-month-old mice. Estradiol proved to be more potent than diethylstilbestrol in eliciting mammary tumors, and triphenylethylene was much less potent than the other two

substances. Of immediate interest here was the low incidence of hepatomas in mice that were 13 to 26 months of age. Of 46 that had carried diethylstilbestrol pellets and were killed between the ages of 13 and 18 months, none had a hepatoma, and of 88 examined between the ages of 19 and 26 months only 10, or 11 percent, had hepatomas. Likewise, of 36 mice in the estradiol-treated group dying when 13 to 18 months old, none had a hepatoma, while of 37 in the 19- to 26-month-old series, 8, or 22 percent, had hepatomas. Of those in the triphenylethylene-treated groups, 2 of 20 in the 13- to 18-month-old group, and 10 of 55 in the 19- to 26-month group had hepatomas. Thus, 282 estrogen-treated C3H males were autopsied between the ages of 13 and 26 months, and 30, or 11 percent, had developed hepatomas. Furthermore, 28 of the tumor-bearing mice were in the 19- to 26-month-old groups. The authors noted that while the only mice of the 13- to 18-month-old group to develop hepatomas had received the weakest estrogen, there was no over-all correlation between the dose of estrogen and the incidence of hepatomas. They also emphasized the lack of untreated controls and concluded "the effect of estrogens on the occurrence of hepatomas in male mice must await further investigation."

The suggestive findings and tentative conclusions of investigators who studied the relationship between hormonal stimulation and the development of spontaneous hepatomas may be summarized as follows. Shimkin and Wyman (*25*) decreased the incidence in intact C3H males by the administration of estrogens, and Miller and Pybus (*24*) obtained similar results with castrated CBA males. These findings appear logical in view of the greater resistance of female mice, but one brief report by Pybus and Miller (*12*) contains the statement "in non-castrated males injected with oestrone there was no diminution in incidence." Burns and Schenken (*16*) and Schenken and Burns (*23*) reported that estrogens increased the incidence in intact C3H males and decreased the incidence in intact females of the same strain. When Schenken and Burns (*23*) treated 48 C3H males and females with testosterone propionate only 1 female developed a hepatoma. It is clear, as indicated by all these investigators, that further efforts along these lines are essential.

In this laboratory attempts to alter the incidence of these tumors by the application of sex hormones have also yielded inconclusive results. This report contains the only significant observation along these lines, namely, that castrated C3H males revealed a lower incidence than did their intact litter mates. Data in table 1 show that the reduction in hepatoma incidence was consistent in 3 series of mice in which litter mates served as controls as well as in 2 other series. However, the evidence is interpreted as suggestive only because one series of castrated CBA males did not show as sharp a decline in incidence and because, as also shown in table 1, the incidence of hepatomas in intact C3H males varied between 12 and 55 percent. Perhaps, as suggested from the data of table 2, castration at a very early age may be necessary to provide conclusive findings. Miller

and Pybus (*24*) found a reduced incidence of hepatomas in CBA males that were castrated at the age of 3 or 4 weeks and given estrogenic stimulation. This problem is receiving attention.

Another very promising avenue of approach to the study of spontaneous hepatomas in mice has been opened recently by the work of Tannenbaum and Silverstone, who found that the diet has a significant influence upon the development of hepatomas in strain C3H animals. Their observations are summarized briefly at this point because they offer an explanation for some of the findings presented here.

In one experiment (*26*), 5 groups of 13- to 14-week-old strain C3H females were fed rations containing 9, 18, 27, 36, or 45 percent casein and 76, 67, 58, 49, or 40 percent cornstarch, respectively: the remaining 15 percent of all diets included identical amounts of other constituents. Ninety-six animals were killed when 61 to 110 weeks old and, depending upon the proportion of casein in their diets, showed hepatoma incidences between 10 and 50 percent: the highest incidence occurred in 18 mice fed the 36-percent-casein diet. Of immediate interest was the presence of hepatomas in 33, or 34 percent, of the female C3H mice, for they showed a much higher incidence than that of 11 percent revealed by the 362 agent-free C3H females of table 1.

In another experiment, 21-week-old C3H males were started on similar diets containing 9, 18, or 45 percent casein and were killed when 13 months of age. Those on 9-, 18-, or 45-percent-casein diets showed hepatoma incidences of 11, 61, and 38 percent, respectively. A total of 135 were killed and, of these, 50 had hepatomas for an over-all incidence of 36 percent. These incidences were comparable to those in 15-month-old males of table 1.

In another paper (*27*) Tannenbaum and Silverstone used C3H males to study the influence of caloric restriction upon the occurrence of hepatomas. In one experiment the diets were initiated when the mice were 8 to 10 weeks old, and 3 weeks later applications of methylcholanthrene to the skin were begun. A total of 229 mice were killed when 62 to 64 weeks of age. Of 70 whose diet was severely restricted in caloric content, none had a hepatoma, whereas 74 whose diet permitted them to develop normally showed an incidence of 55 percent. One group of 36 mice received the most calories, showed the highest body weight (35 to 40 gm.), and the highest incidence (64 percent) of hepatomas. In another experiment 13- to 14-week-old mice were started upon diets to ascertain the effect of under-feeding upon the development of hepatomas. These animals were killed when 58 to 59 weeks old. Fifty mice fed a daily ration of 3.8 to 4.0 gm. attained body weights of 37 to 40 gm. and had a hepatoma incidence of 44 percent, whereas of 21 underfed mice none developed a hepatoma.

In the 3 experiments summarized above, Tannenbaum and Silverstone used 221 strain C3H males that attained body weights of 35 to 40 gm. and were killed when 52 to 64 weeks of age. These showed hepatoma incidences of 11 to 64 percent, with an over-all incidence of 43 percent. It

would be of interest to know the incidence of hepatomas in their C3H mice, especially in the females, if the animals were maintained on a standard or commercial diet. The only suggestion along these lines is found in a paper by Silverstone (*28*), which records the presence of hepatomas in 3 of 19 males killed at 13 to 14 months of age after ingesting a diet of Purina fox chow.

The investigations of Tannenbaum and Silverstone show that in any efforts to alter the incidence of hepatomas in mice the experimental and control animals must receive identical diets.

As stated previously, all mice used in the present studies were fed a ration of either Purina dog chow or Purina laboratory chow, but the diet was not changed during the course of an experiment. Diet may have been responsible for some of the results shown in table 1, because Series 3 males were fed dog chow and showed hepatoma incidences of 0 to 23 percent, while Series 5 males ingested laboratory chow and showed incidences of 18 to 30 percent. However, confirmation of this observation was not possible because males of Series 3 were the only animals listed in the table that consumed the dog chow: all others ate the laboratory chow.

SUMMARY

Totals of 1,150 strain C3H and 212 strain CBA mice were used in studies on the occurrence of spontaneous hepatomas.

Mice of both strains developed hepatomas but the tumors occurred more frequently in the males.

The tumor incidence in different groups of normal C3H males varied between 12 and 55 percent. There was no significant difference between the over-all tumor incidences in breeding and virgin C3H males.

Castrated C3H males showed lower incidences than did their intact litter mates. The over-all incidence of hepatomas in castrates was also lower than the over-all incidence in breeders and virgins.

These findings are discussed in relation to published accounts of efforts to alter the incidence of spontaneous hepatomas in these strains by the administration of hormones or by the use of experimental diets.

REFERENCES

(*1*) Murray, J. A.: Spontaneous cancer in the mouse. Third Scientific Report, Imperial Cancer Research Fund, London, p. 98. (1908).

(*2*) Slye, M., Holmes, H. F., and Wells, H. G.: Spontaneous primary tumors of the liver in mice. J. Med. Research 33: 171–182 (1915).

(*3*) Strong, L. C.: The establishment of the C3H strain of mice for the study of spontaneous carcinoma of the mammary gland. Genetics 20: 586–591 (1935).

(*4*) ———: Production of the CBA strain of inbred mice; long life associated with low tumor incidence. Brit. J. Exper. Path. 17: 60–63 (1936).

(*5*) ———: The origin of some inbred mice. Cancer Research 2: 531–539 (1942).

(*6*) Strong, L. C., and Smith, G. M.: Benign hepatomas in mice of the CBA strain. Am. J. Cancer 27: 279–284 (1936).

(*7*) Strong, L. C., Smith, G. M., and Gardner, W. U.: Induction of tumors by 3:4:5:6—dibenzcarbazole in male mice of the CBA strain, which develops spontaneous hepatoma. Yale J. Biol. & Med. 10: 335–346 (1938).

(8) STRONG, L. C.: The incidence of spontaneous tumors of mice of the CBA strain after a change of diet. Am. J. Cancer 32: 80–84 (1938).

(9) ———: The incidence of spontaneous tumors in female mice (breeders) of the CBA strain. Am. J. Cancer 32: 85–89 (1938).

(10) GORER, P. A.: Seventeenth Annual Report. British Empire Cancer Campaign, p. 232 (1940).

(11) ———: The incidence of tumors of the liver and other organs in a pure line of mice (Strong's CBA strain). J. Path. & Bact. 50: 17–24 (1940).

(12) PYBUS, F. C., and MILLER, E. W.: Nineteenth Annual Report, British Empire Cancer Campaign, p. 42 (1942).

(13) MILLER, E. W., and PYBUS, F. C.: The inheritance of cancer in mice with special reference to mammary carcinoma. Cancer Research 5: 84–93 (1945).

(14) ANDERVONT, H. B.: The occurrence of spontaneous and induced pulmonary and liver tumors in strain C3H mice. Pub. Health Rep. 54: 1158–1169 (1939).

(15) ANDERVONT, H. B., and MCELENEY, W. J.: Spontaneous tumors in a subline of strain C3H mice. J. Nat. Cancer Inst. 1: 737–744 (1941).

(16) BURNS, E. L., SCHENKEN, J. R.: Spontaneous primary hepatomas in mice of strain C3H. A study of incidence, sex distribution and morbid anatomy. Am. J. Cancer 39: 25–35 (1940).

(17) ———: Spontaneous primary hepatomas in mice of strain C3H. II. The influence of breeding on their incidence. Cancer Research 3: 691–692 (1943).

(18) ANDERVONT, H. B., and DUNN, T. B.: Effect of castration and sex hormones on the induction of tumors in mice by *o*-aminoazotoluene. J. Nat. Cancer Inst. 7: 455–461 (1947).

(19) EDWARDS, J. E., DALTON, A. J., and ANDERVONT, H. B.: Pathology of a transplantable hepatoma in a C3H mouse. J. Nat. Cancer Inst. 2: 555–563 (1942).

(20) DALTON, A. J., and EDWARDS, J. E.: Mitochondria and Golgi apparatus of induced and spontaneous hepatomas in the mouse. J. Nat. Cancer Inst. 2: 565–575 (1942).

(21) ANDERVONT, H. B., and DUNN, T. B.: Mammary tumors in mice presumably free of the mammary tumor agent. J. Nat. Cancer Inst. 8: 227–233 (1948).

(22) ANDERVONT, H. B.: Incidence of mammary tumors in mice of strain C3H and in descendants of fostered strain C. J. Nat. Cancer Inst. 10: 193–200 (1949).

(23) SCHENKEN, J. R., and BURNS, E. L.: Spontaneous primary hepatomas in mice of strain C3H. III. The effect of estrogens and testosterone propionate on their incidence. Cancer Research 3: 693–696 (1943).

(24) MILLER, E. W., and PYBUS, F. C.: The effect of oestrone in mice of three inbred strains, with special reference to the mammary glands. J. Path. & Bact. 54: 155–168 (1942).

(25) SHIMKIN, M. B., and WYMAN, R. S.: Mammary tumors in male mice implanted with estrogen-cholesterol pellets. J. Nat. Cancer Inst. 7: 71–75 (1946).

(26) TANNENBAUM, A., and SILVERSTONE, H.: The genesis and growth of tumors. IV. Effects of varying the proportion of protein (casein) in the diet. Cancer Research 9: 162–173 (1949).

(27) ———: The influence of the degree of caloric restriction in the formation of skin tumors and hepatomas in mice. Cancer Research 9: 724–727 (1949).

(28) SILVERSTONE, H.: The effect of rice diets on the formation of induced and spontaneous hepatomas in mice. Cancer Research 8: 309–317 (1948).

TRANSPARENT-CHAMBER OBSERVATIONS OF THE RESPONSE OF A TRANSPLANTABLE MOUSE MAMMARY TUMOR TO LOCAL ROENTGEN IRRADIATION [1]

RUTH MERWIN and GLENN H. ALGIRE, *National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.*, and HENRY S. KAPLAN, *Department of Radiology, Stanford University School of Medicine, San Francisco 15, Calif.*

INTRODUCTION

The effects of ionizing radiation on malignant neoplasms are generally attributed both to a direct action on the tumor cells and to an indirect action on them mediated by the blood vessels and connective tissues of the tumor. There is controversy over the relative importance and precise roles of these respective elements. The transparent chamber technique offers a method for *in vivo* observations of the behavior of a tumor after irradiation, making possible some degree of separation of the role of various factors.

It is important to recognize that the direct and indirect effects of irradiation may fluctuate in magnitude according to type and size of tumor and with the dose of irradiation. Observations have been made on a transplantable mammary adenocarcinoma after local irradiation with a dose that caused distinct regression but permitted recovery of the tumor tissue within the period of time a chamber could be maintained after irradiation (approximately 5 weeks).

MATERIALS AND METHODS

The transparent chamber technique (*1*) was used in making microscopic observations of transplanted tumor fragments. The implant lies upon the striated muscle layer found beneath the skin. Between the tumor and the mica window of the chamber there is only a thin layer of connective tissue.

The tumor used was a spontaneous mammary adenocarcinoma (C3HBA) from a strain C3H mouse (*2*).[2] Implants varied from about 0.2 to 1.1 sq. mm. in area. The source tumor material varied in age, size, and consistency.

[1] Received for publication November 24, 1950.

[2] We should like to express our appreciation to Dr. Morris K. Barrett of the National Cancer Institute for making available this tumor during its 39th to 61st transfer generations (over a period of 14 months).

Strain C3H mice, mostly males, 2½ to 5½ months of age were used. They were kept in individual transparent plastic cages in a constant temperature incubator maintained at 30° C. Purina laboratory chow and water were always available.

Roentgen irradiation was administered to a circular area of the window 1 cm. in diameter while the rest of the mouse was carefully shielded.[2] A single dose of 2,000 to 5,000 roentgens was delivered.

Daily microscopic observations were made, including measurements of the tumor implants with an ocular micrometer, and frequent photomicrographs were taken. Tumor thickness was measured by means of calipers when it exceeded 1 mm. Histologic preparations of the tumors and surrounding tissues were made when the windows were discarded but a detailed discussion of the histologic observations is not presented here.

The growth rate is calculated in terms of K, the log of the proportional increase in tumor volume per unit of time, as described by Blum (*3*). Since volume is based here upon only two dimensions, Blum's formula 12 was used. The value of K, unless otherwise stated, was calculated from a growth curve fitted to the observed points for each individual tumor by the method of least squares. For average growth rates each separate growth rate was weighted by the accuracy with which it was determined, and an over-all weighted average growth rate found. Some K values, as indicated in the tables, were estimated by the use of a specially constructed scale that gave the slope of a line fitted to the observed points by eye.

Three types of experimental observations were made:

(A) The growth of implanted tumor fragments was observed for a varied period of time before local irradiation was administered and as long as possible thereafter.

(B) Nonirradiated tumor tissue was implanted in a window irradiated from a few minutes to 10 weeks previously.

(C) Tumor tissue was irradiated, and after an interval of a few minutes to 6 days was placed in a nonirradiated window. Tumor tissue regrowing after irradiation was also transferred to nonirradiated windows.

OBSERVATIONS AND RESULTS

(A) Tumor Implants Observed Before and After Irradiation *in Situ*

1. *Behavior of tumors before irradiation.*—In these experiments vascularization had begun by 2 to 8 days after implantation (average 5.2 days). Details of the vascularization in the transparent chamber in tumors of this type have been described by Algire and Chalkley (*4*) and will not be reported here.

The percentage increase in area per day is recorded in table 1 wherever 3 or more reliable measurements were made. The average value of K for 22 tumors was 0.214 with a standard error of $\pm .0016$ and a range of 0.141 to 0.333. Thus, on the average, the tumors doubled in volume in

[2] Physical factors were as follows: 186 KVP, 170 v., and 20 ma., 800 r per minute at a focus skin distance of 14.5 cm. through 0.25 mm. Cu and 0.55 mm. Al filters.

TABLE 1.—*Changes in tumor implants irradiated* in situ

Experiment No.	Percent increase in area per day prior to irradiation	Number of days—		Area when irradiated in sq. mm.	Circulation			Percent change in area after 1 day	Percent change in area—	Number of days—	Number of days after irradiation		
		Tumor implanted before irradiation	Tumor vascularized before irradiation		When irradiated	After 1 day	After 3 days		Between 1 day after irradiation and last day meas. were obtained during regression [2]		Before regrowth detected		Before mouse discarded with no sign of regrowth
											Window in	Window out	
5,000 r													
1	62	16	10	15.0			Slower	−18	−42	11			
2a [4]	[1] 55	8	5	11.0	Slow	Slow		−14	−45	8		37	
2b [4]	[1] 51	8	5	6.8				−9	−47	8			
3	[1] 48	13	5	6.0	Good	Rapid	Slow	0	−32	3			124
4		14	7	4.8	do	Good	do	+4	−12	7			27
5	[1] 32	8	4	3.5	Poor							21	
3,000 r													
6	[1] 66	14	6	27.0	Slow			+11	−23	3		15	
7	[1] 41	20	[5] 6	22.2		Slower	Slow	+5	+24	7	9		
73 [3]	[1] 38	10	4	19.4				+27	−2	5	10		
9	59	11	7	13.5			Slow	+30	−40	21	23		
10	[1] 41	9	4	6.9	Rapid			+10	−8	4	20		
11 [4]	41	8	5	5.6	Slow	Faster	Slow	+17	−47	16	17		
12	41	9	3	5.0				+12	−42	5			
13	[1] 45	9	4	4.3			Rapid	0			15		
14	[1] 59	14	8	3.7				+8	+18	3			
15	29	9	5	2.5				+12	−57	14			327
16	[1] 32	9	4	2.4	Slow			+4	+4	6			
17	48	8	3	2.0				+13	−22	12	21		
18	[1] 45	6	4	1.9	Good	Good	Slow	0	−32	14			
19 [4]		8	0	1.6		Present		0					54
20		6	2	1.5				+8	−46	7			
21 [4]	[1] 29	8	3	1.2		Good	Slow	0	−17	6		25	
22	35	8	4	.8		Slow	do	−6	−29	6		32	
23		6	1	.2	Good	Faster	Slower	0					21

TABLE 1.—*Changes in tumor implants irradiated* in situ—Continued

Experiment No.	Percent increase in area per day prior to irradiation	Number of days—		Area when irradiated in sq. mm.	Circulation			Percent change in area after 1 day	Percent change in area—	Number of days—	Number of days after irradiation		
		Tumor implanted before irradiation	Tumor vascularized before irradiation		When irradiated	After 1 day	After 3 days		Between 1 day after irradiation and last day meas. were obtained during regression [2]		Before regrowth detected		Before mouse discarded with no sign of regrowth
											Window in	Window out	
						2,000 r							
24	[1] 38	9	5	15.1			Slow	+16	−33	3			
25	{ 15 / [6] 59 }	22	15	8.6	Rapid								
26	[1] 51	11	5	6.8	Slow		Slow	+13	−10	9	15		
27	[1] 29	8	3	4.6			do						
28	45	8	4	3.2	Good	Good							
29	[1] 29	7	4	2.9	Slow	Slow		+7	−32	13	13		
30a [7]	41	8	4	2.4				0	−17	4			
31a		8	3	2.3				+32	0				
32		8	4	1.9				+8	−4	4			
33		8	2	1.6				+19	−21	7	9		
34		6	2	1.6	Slow	Slow	Slow	+33	−36	14	14		
35		8	3	1.5		Faster	Slower						
30b	45	8	4	1.4				+21	−32	4			
31b		8	3	1.2				−11	+4	3			
36		6	2	1.1	Slow			+12	−14	18	18		
37		8	4	.8	Good								
38		8	2	.4		Rapid		−6	−74	14	22		

[1] Based on K calculated by the method of least squares, others are based on K determined by the use of a special scale.
[2] The last measurement obtained during regression was the last one before the window was removed, or before regrowth was detected or before a host cellular layer became marked.
[3] See table 5, implant was from tumor regrowing after irradiation.
[4] Window in a female mouse.
[5] Tumor was not vascularized until 14 days after transplantation because it was stuck to the mica window of the chamber.
[6] Tumor grew at the slower rate for 6 days and at the faster for 5 days.
[7] 2 tumors in the same window are designated a and b.

1.4 days. The rate of growth remained essentially constant except in a few tumors (experiment 25, table 1). The growth rate of larger tumors remained constant despite the fact that their vessels were flattened to a ribbonlike thinness after the tumors exceeded an area of about 7.0 sq. mm.

A detailed study of vessels in a growing tumor showed that between any two functioning vessels there were small capillaries through which only an occasional corpuscle passed and there were strands of endothelium in which no lumen could be distinguished. So many vascular changes took place from one day to the next that it was difficult to trace individual vessels, but it seemed probable that as the tumor cells multiplied and thus increased the distance between functioning capillaries, the small vessels enlarged and the endothelial strands became functioning vessels.

Forty-one tumors ranging in area from 0.2 to 27 sq. mm. were each irradiated *in situ* in 38 windows at intervals of 6 to 22 days after transplantation, 1 to 15 days after vascularization.

2. *Behavior of tumors after irradiation* in situ.—Observations are for clarity of presentation placed in three groups: (a) changes during the first day after irradiation; (b) changes during regression; (c) changes during regrowth.

(a) *Changes during the first day after irradiation.*—By 24 hours after irradiation a slight general opacity of the tumor tissue could usually be detected.

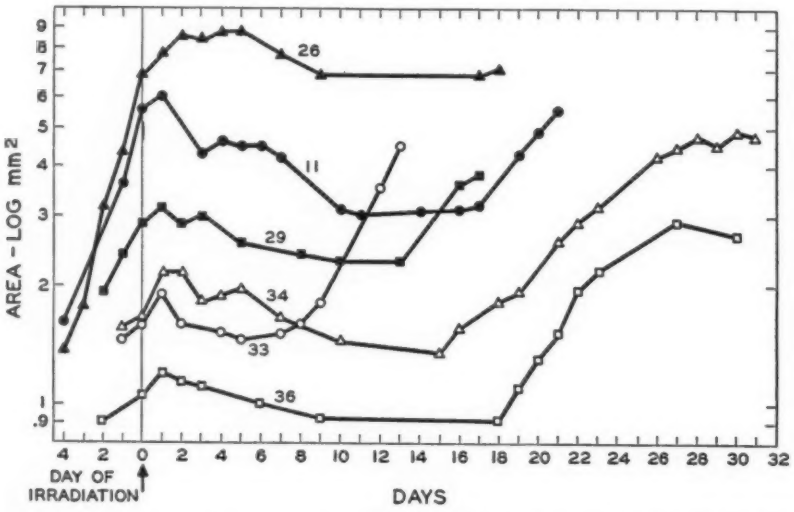
Vessel narrowing was conspicuous in the small tumors. A less conspicuous narrowing, detected only on comparing photographs, was also present in some larger tumors (figs. 1 and 2) but was delayed in others. In implants or portions of implants where no vessels were visible at the time of irradiation, functional vessels sometimes appeared in 1 to 3 days (figs. 3 and 4).

The circulatory rate before irradiation varied (table 1). It seemed to depend on the number and type of vascular connections made with host vessels. Although the circulation was slower in some implants 1 day after irradiation, in others it was more rapid than it ever became in nonirradiated tumors. The rapid flow was apparent by 6 hours and was still present at 24 hours.

Changes in tumor areas are given in table 1 and some are presented graphically in text-figure 1. The average increase in area per day prior to irradiation was 42 percent. Considering together those tumors given 2,000 r or 3,000 r there was an average increase during the first day after irradiation of 11 percent, due in part to a flattening of the tumor (table 2). The few tumors given 5,000 r showed an average decrease in area of 7 percent.

(b) *Changes during regression.*—The period of regression was of variable duration. It began 1 day after irradiation when the tumor started to decrease in volume and ended when growth was resumed. The regression of 13 tumors was followed until regrowth began; 27 others had to be discarded before this, usually because of the accumulation of host cells in the window.

922686—51——16



TEXT-FIGURE 1.—Area changes after irradiation of tumors irradiated and left *in situ*

TABLE 2.—*Comparison of changes in diameter of tumor in the plane of the mica window with changes in thickness of the tumor* [1]

Experiment No.	Percent increase per day before irradiation			Percent change by 1 day after irradiation			Percent change between 1 and 2 days after irrad.			Percent change between 1 and 3 days after irrad.		
	Average diameter	Thickness	Volume	Average diameter	Thickness	Volume	Average diameter	Thickness	Volume	Average diameter	Thickness	Volume
6	[2] 29	39	123	7	−2	5	−10	−28	−39	−13	−35	−48
7	[2] 24	[2] 23	[2] 108	2	−5	0	4	−17	−15	10	−17	−2
9	[2] 26	----	----	11	2	14	−6	−22	−32	−13	−22	−39
11	----	----	----	2	−7	−1	----	----	----	----	----	----
24	[2] 17	10	67	9	0	17	----	----	----	−21	−9	−39
26	[2] 23	11	76	6	0	15	6	−20	−13	5	−23	−17
39	[2] 14	[2] 16	[2] 59	----	----	----	----	----	----	----	----	----
40	[2] 12	[2] 19	[2] 55	----	----	----	----	----	----	----	----	----

[1] An error is introduced in thickness measurements because the skin must be included. No correction for skin thickness has been made.
[2] Based on three measurements or more.

The degree and rate of changes in tumor size varied greatly (tables 1 and 2). Where both area and thickness measurements are available for a few larger tumors, they indicate that the volume increased slightly at 1 day but had decreased by 2 days. There was some flattening during the first few days after irradiation, so that area and volume changes were not the same. In many tumors the rate of decrease in area was rapid for a week, after which it slowed. Although micrometer measurements pre-

sented in text-figure 1 suggest that some tumors stopped shrinking after 10 days, measurements on photographs show that a slow decrease in area continued until regrowth obscured further changes.

The degree of opacity of the implant often increased for a few days following irradiation but in a week a gradual decrease had begun. Whether the circulation was rapid or slow 1 day after irradiation it was usually slow by 2 to 4 days. The circulation improved following a general narrowing of vessels. This narrowing had by 5 to 7 days after irradiation produced a venous drainage system that showed a treelike pattern with small vessels emptying into successively larger ones. In nonirradiated material there was often little relation between size of vessel and direction of flow except in the largest veins. As the vessels narrowed the tumor lost its conspicuous redness and became almost indistinguishable in color from the surrounding tissue.

(c) *Changes during regrowth.* Observations were made on 12 tumors in which regrowth began 9 to 23 days after they had received a dose of 2,000 to 3,000 roentgens. In the 3 windows containing tumor tissue that was not vascularized at the time of irradiation this tissue was the first to start regrowing. Regrowth became evident with the appearance of 1 or more foci in which tumor tissue was more translucent and blood vessels were enlarged. Most of the foci were 0.1 to 0.3 sq. mm. in area when first detected (table 3).

The following changes took place in the foci during the first 5 days. The vessels increased in caliber but not in number (figs. 5 and 10–12) and since the tumor tissue continued to grow the intercapillary distances became greater. Measurements of the growth of whole foci or of the increase in individual intercapillary distances made in succeeding photographs show that the rate of regrowth was equal to that of nonirradiated tissue (text-fig. 2). Four growth curves, each with at least 3 points obtained during the first 5 days showed an average K value of $0.217 \pm .0025$, which is not significantly different from the average normal rate prior to irradiation.

A slowing of the growth rate began about 5 days after the foci were detected, apparently due to a deficient blood supply that developed as tumor-cell growth increased the distance between vessels. The vessels ceased to enlarge and began to narrow (fig. 13) and small opaque areas appeared at points remote from vessels, again suggesting the inadequacy of the circulation. Vascular stasis developed, followed by a fragmentation of vessels (fig. 14), and the entire regrowth area thus deprived of a vascular supply become more opaque (fig. 14) and in some places necrotic (fig. 16). Only the tissue on the periphery of the regrowth area was near functioning vessels, and these vessels became enlarged. Subsequently nonirradiated capillaries grew in toward the tumor implant from the nonirradiated portion of the skin. The events leading to the ingrowth of these vessels and the significance of their formation with respect to the final fate of the regrowth foci are discussed in connection with experiments reported later in this paper.

TABLE 3.—*Regrowth of tumors irradiated and left* in situ

3,000 r

Experiment No.	Site of regrowth not vascularized at time of irradiation	Approximate size of regrowth foci when first detected in sq. mm.	Number of days after irradiation before regrowth foci detected	Number of days after regrowth detected before opaque areas appeared	*K* value for regrowth rate of entire tumor	Days after irradiation for which the *K* value is given	Comments
7	------	------	9	------	------	------	Marked cellular reaction.
8	------	------	10	5	------	------	" " "
9	------	0.2	23	7	0	23–39	Scattered foci embedded within implant.
	------	.3		7			
	------	.2	24	7			
	------	.2	28	7			
10	(2)	.4	[3] 20	<8	------	------	Regrowth focus in a separate little tumor which was vascularized during 1–3 days after irradiation.[1]
11	------	.2	17	------	.09	17–21	Foci scattered through tumor.
	------	.2		------			
	------	(4)	18 or 19	------			
13	(2)	------	15	6	------	------	See comment for experiment 10.[1]
17	(2)	.3	21	4	------	------	Regrowing focus in portion of implant adjoining main tumor, a portion vascularized 1–3 days after irradiation.[1]

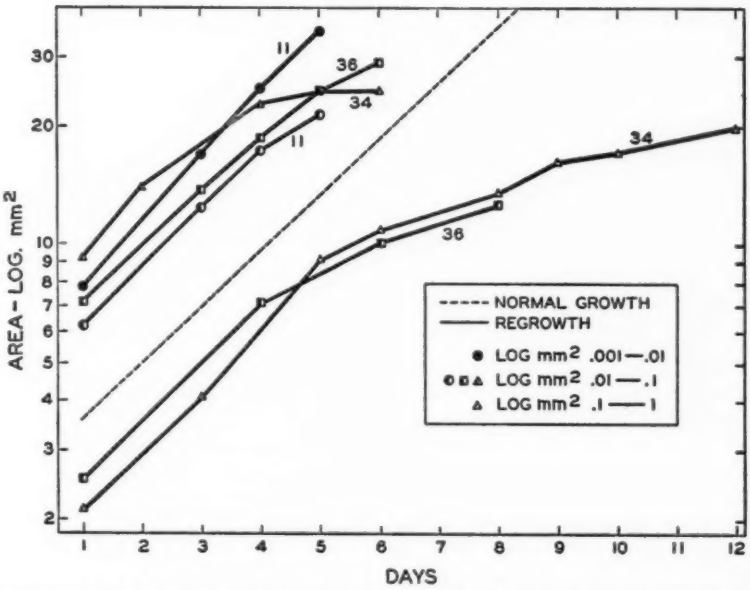
2,000 r

26	------	0.7	15	------	0	15–17	Foci scattered through tumor.
	------	.2		------			
	------	(4)	17	------			
29	------	(4)	12 or 13	------	.07	13–17	" " " "
	------	(4)	14	------			
33	------	(4)	9	------	.15	9–13	" " " "
34	------	.5	14	7	.07	15–26	" " " "
	------	.1	16	12	.01	26–31	
	------	.1	17	13			
	------	.1	22	------			
36	------	(4)	18	5–8	.12	18–23	" " " "
					.03	23–27	

[1] Regrowth was observed in experiment 10 for 13 days, and in experiments 13 and 17 for 19 days before the window was removed but there was too much cellular reaction to obtain growth records.
[2] Site not vascularized.
[3] Vessels never were fine so that it was hard to tell when enlargement due to regrowth started.
[4] Many foci appeared on the same day.

When several foci started growing within a small implant the growth rate for the entire tumor was temporarily within the range for nonirradiated tumors (table 3). However, some tumors containing several foci showed no increase in size.

A few mice in which the window was removed during regression were kept to see if and when there would be a "recurrence" of the tumor. Regrowth was detected in some between 15 and 37 days after irradiation but none was detected in others kept over 40 days (table 1).



TEXT-FIGURE 2.—Growth of localized areas within a tumor following irradiation. Measurements were made at any time after regrowth began when growth could be followed by means of measurements between blood vessels recognizable in succeeding photographs. For all except the top curve measurements were started within 2 days after regrowth was first detected.

(B) BEHAVIOR OF NONIRRADIATED TUMORS IN IRRADIATED WINDOWS

Tumor implants were placed on an irradiated bed to ascertain whether irradiated capillaries could produce new vessels. If reports in the literature (*5*) and our conclusions, that vessels in regrowth foci could not produce new ones, are correct, no vessels should have been produced. The behavior of embryonic tissue also placed on an irradiated bed is reported because it demonstrated the role in vascularization played by the vessels of the implant itself. Fifteen windows were each given a single dose of 2,000 or 3,000 roentgens 15 minutes to 75 days before implantation of the tumor or embryonic fragments. Physical factors were as previously described.

In these experiments any vessels appearing in an implant before 5 days after transplantation are thought to be implant vessels that have established a functional connection with host vessels, for it has been our experience with nonirradiated windows [unpublished; *cf.* Clark (*6*)] that new functional vessels from the host do not usually appear until 5 to 8 days after a stimulus is applied. The responses obtained are recorded in table 4. A rich network of vessels appeared in less than 5 days in most embryonic implants indicating that the embryonic vessels developed a functional

TABLE 4.—*Behavior of nonirradiated implants in an irradiated window*

A.[2] Embryonic implants

Experiment No.	Dose in r	Days between—		Source of implant endothelium	Observations on vascularization	Observations on growth of tumor	Growth *K* value	Days after transplantation covered by *K*
		Irradiation of window and implantation	Implantation and vascularization					
41	3,000	0	3	Implant itself	Rich network of vessels			
42	3,000	0	2					
43	3,000	0	2					
44	3,000	0	3					
45	2,000	5	5					

B. Tumor implants

46	3,000	0	2	Probably [1] implant itself.	Few vessels, some of the earlier ones disintegrated.	Vessels became enlarged and tissue farthest from vessels opaque.	0.11	11–29
47	3,000	0	2				.16	14–20
48	2,000	4	10				.08	13–22
49	2,000	75	3–5					
50	3,000	0	26	Nonirradiated area of host.	Vessels grew into the irradiated area from the nonirradiated zone through a host formed layer of cells.	Vessels normal		
51	3,000	0	27					
52	3,000	0	21					
53	2,000	0	12			Vessels normal	.15	22–26
54	3,000	0	19	Nearby embryonic implant.	Vessels of embryonic implant grew into host cellular layer and reached tumor implant.	Vessels normal		
55	3,000	0	12		No vascularization of implant before window removed.			
56	3,000	0	9					
57	2,000	6	10					

[1] See text.
[2] Embryonic thyroid.

contact with host vessels. Some tumor implants were vascularized by a few vessels during the 2d to 5th days after transplantation, just as some tumor implants on a nonirradiated bed are; but no rich network of vessels appeared in 5 to 8 days after implantation as it does on a nonirradiated bed. We may conclude that in tumor transplants only a few or none of the implant vessels made a functional contact with host capillaries and that, except in two questionable instances, the host-irradiated endothelium did not produce new vessels. In one instance a richer network of vessels appeared 15 days after transplantation but it may have been formed by the proliferation of the implant vessels. In the other instance the first vessels appeared at 10 days after transplantation, but it seems likely that they were surviving implant vessels for they showed disintegration and hemorrhage just as did vessels appearing in implants within 5 days after transplantation.

All the vessels in the tumor implants were probably implant vessels. They behaved differently from the irradiated vessels in regrowing tumors left *in situ*, for they reached a larger caliber (fig. 19), they did not fragment as growth continued, and in one case there was evidence that they produced new vessels (fig. 20); the inability of the rest of the vessels to increase in number may have resulted from the poor environmental conditions.

Host cells accumulated between the mica and the tissue around these tumor implants on an irradiated bed. They accumulated earlier around tumors in which no implant vessels were functioning. The layer of host cells became continuous over the entire window, including both the irradiated and nonirradiated zones. Layers of this type may occur in chambers whether or not an implant is present in both nonirradiated and irradiated windows but more often in the latter, apparently in response to various irritating stimuli. After such a layer had been in contact with normal vessels for about a week, capillaries appeared in it. New vessels never developed in the irradiated zone of the windows possessing such a layer but grew into the layer from the surrounding nonirradiated area and thus reached the implant.

After vessels from a nonirradiated zone reached the nonirradiated tumor tissue, the tumor growth-rate was within the normal range. When the endothelium of the tumor implants survived and functioned it was usually not able to support the normal rate of growth.

These observations of nonirradiated tumor implants placed in irradiated windows aid in interpreting postirradiation changes in a tumor left *in situ* in that they 1) provide further evidence that irradiated endothelium cannot produce new vessels, and 2) indicate more clearly than did observations of the regrowth foci the events leading up to the invasion of the irradiated area by nonirradiated vessels. The importance of this invasion in relation to the fate of regrowth foci is brought out in the next section.

(C) Behavior of Irradiated Tumors in Nonirradiated Windows

Irradiated tissue was placed in nonirradiated windows to see whether regrowing tumor tissue would behave normally when provided with nonirradiated vessels, for tissue regrowing *in situ* showed abnormalities that seemed to be caused by the inability of the irradiated endothelium to grow. Of 16 windows implanted with irradiated tumor tissue 12 received fragments irradiated 15 minutes to 5 days previously and 4 received tissue already regrowing after irradiation (table 5).

Owing to a confusion of cellular reaction and host vessels surrounding the implants, we were not able to follow the details of vascular and opacity changes at the time of regrowth to see whether they resembled those observed in regrowth *in situ*. However, we were able to measure growth rates. The regrowth of the one focus followed over 7 days did not slow down as it did in regrowth foci of tumors left *in situ*. One other tumor also continued to grow at the normal rate longer than 7 days after regrowth began; it was a tumor that did not become vascularized until a week after regrowth had been initiated.

Implants of tumor tissue regrowing at the time of transplantation grew in the same manner as implants of nonirradiated tumor. Thus, whether the tumor tissue was regrowing before it was transferred or started regrowing afterwards, it behaved essentially like nonirradiated tumor tissue. This demonstration that regrowing tumor tissue grows at the normal rate if supplied with nonirradiated vessels suggests that if regrowth foci of tumors left *in situ* were to become vascularized by nonirradiated vessels a normal rate of growth could be expected.

It was observed in earlier experiments where tumors were irradiated and left *in situ* that the vessels narrowed after irradiation. Although the decrease in caliber of vessels seemed to be secondary to the cessation of tumor-cell growth, there was the possibility that vessels narrowed as a direct response to irradiation. A nonirradiated window containing an irradiated implant was found in which the secondary nature of the fluctuations in vessel caliber, both the narrowing after irradiation and the widening at the time of regrowth, was shown. Dilatation and tortuosity of vessels underlying the slightly opaque implant developed in a few days. These vessels gradually subsided to a normal capillary caliber in 2 weeks, while the implant became more translucent. On the 27th day after transplantation a slight opacity developed in the tumor tissue, and the underlying vessels again enlarged. The size and opacity of the implant increased for 7 days before functional host vessels appeared in it, followed by a change to greater translucency and a normal pattern of growth in the tumor.

TABLE 5.—*Behavior of irradiated tumors in nonirradiated windows*

Experiment No.	Source of tumor tissue	Dose in r	Implant area in sq. mm.	Interval in days between—			Regrowth K value	Days after regrowth initiated covered by K [1]	Number of days between irradiation and removal of window	Comments
				Irradiation and transplantation	Transplantation and vascularization	Irradiation and regrowth				
58	Fragment from locally irradiated subcutaneous tumor.	3,000	0.45	0	4	17	------	--------	28	Severe cellular reaction.
59		3,000	.36	0	6	86–196	------	--------	46	Regrowth began after window removed.
60		2,000	------	0	[2]	-------	------	--------	41	Vascularized after regrowth began.
			------	0	34	27	0.20	9–13		
61		2,000	------	0	[2]	43	------	--------	28	Regrowth began after window removed.
			.03	0	10					
62		2,000	.78	0	4	49	------	--------	25	" " " " "
63	Whole tumor from another window.	2,000	7.7	5	2	15–21	------	--------	25	Center never vascularized, opaque; severe host cellular reaction.
64		2,000	6.9	6	20	35	------	--------	36	
65		2,000	3.9	5	5	35	------	--------	16	Regrowth began after window removed.
66		2,000	2.0	4	2	18	.22	6–9	27	
67		2,000	1.9	4	2	19–24	------	--------	28	Several areas began to regrow on different days.
68		2,000	1.2	5	3	18	------	--------	21	
69		2,000	1.1	4	7	17	.18	1–4	28	Severe cellular reaction.
70	Fragment from regrowing tumor.[3]	3,000	1.08	34	4	12	.28	27-31	43	
71		3,000	.55	34	4	12	.22	27–31	43	
72		3,000	.36	34	5	12	.14	27-31	43	
73		3,000	.36	34	5	12	.22	27–31	43	

[1] K calculated by method of least squares.
[2] Not vascularized.
[3] Tumor was irradiated for another experiment on two occasions in one host and three times in a second host during a period of 2 months; irradiation was carried out in each case several days after regrowth was noted and implantation was performed 34 days after the last irradiation at a time when regrowth had been evident for 12 days.

922080—51——17

DISCUSSION

Although it is generally agreed that irradiation has both direct and indirect effects on tumor cells, opinions differ widely regarding the relative importance of these effects. Opinions also differ about the role of various indirect factors such as connective tissue changes and blood vessel changes in the initial response or in the recovery of tumors (*7–13*).

The changes observed during the present investigation in tumors irradiated and left *in situ* are briefly summarized. It should be remembered that the interacting factors may be different in other types or sizes of tumors or with other radiation techniques.

The tumors increased in size during the first day after irradiation but decreased thereafter. By 1 day after irradiation some decrease in the caliber of vessels and a slight opacity was evident; by 2 to 4 days the circulation was often slowed and the degree of opacity often greater; by 1 week the vessels had narrowed greatly and a slow decrease in the degree of opacity had begun.

When tissue that was not vascularized at the time of irradiation was present in a window it was the first to start regrowth. Regrowth was initiated by the appearance of foci growing at the same rate as nonirradiated implants. Vessels within the foci enlarged, but no new vessels were produced. The rate of growth slowed, vessels narrowed and fragmented, and the tumor tissue became more opaque. A host cellular layer formed and vessels from the nonirradiated area growing through this layer approached the tumor.

The increase in size during the first day after irradiation may have been the result of edema, often reported after irradiation. The subsequent decrease in size was attendant on the narrowing of vessels, on the inhibition of mitosis, and probably on the degeneration of cells. We have shown that narrowing of vessels followed as a consequence of the cessation of tumor growth and was not the result of a direct action of irradiation on the vessels.

The degree of opacity we observed during the first few days after irradiation should be considered in relation to the number of degenerating cells reported by Lasnitzki (*9*) in a transplantable mammary adenocarcinoma in mice after a similar dose of irradiation. She found that degenerating cells had become more numerous by 1 day after irradiation, and that a peak was reached at 4 days. Her *in vivo-in vitro* comparisons of the number of degenerating cells indicated that beginning at 2 days there was some indirect action. Since vascular injury also began at 2 days the indirect action was attributed to it. Lasnitzki's findings suggest that the slight general opacity we observed at 1 day was the result of a direct response of the tumor cells, and that the increased opacity that developed over the next few days may have been in part an indirect response. Although we observed no vascular injury, the slow circulation may have acted as an indirect factor.

When nonvascularized tumor tissue was present in a window it was the first to regrow. Mottram (*14*) reported a related phenomenon in tar warts exposed to superficial roentgen irradiation; histologic study showed that

cells close to vessels were most damaged. It is well known that a tumor with poor circulation is less responsive to treatment (*15*). On the other hand, a poor circulation has not in all cases resulted in a protective effect (*16*). In our experiments, although the tissue that was not vascularized at the time of irradiation regrew before vascularized tissue in the same window regrew, when different windows were compared the nonvascularized tissue did not start regrowth sooner than well vascularized implants in other windows. Further investigations will be necessary to ascertain whether the circulatory environment is the main factor causing the variation in response to irradiation.

Our observations of regrowth on an irradiated bed and those of Goldfeder (*17*) on a nonirradiated bed show that the recovery of tumor cells is complete, not partial, nor is it slow, as some have suspected. Slowing in regrowth rate of a tumor as a whole, as well as of individual foci, we found to be due to vascular abnormalities that developed as a result of the inability of irradiated endothelium to grow, an inability also demonstrated by Takahashi (*5*) in histologic studies of the effects of radium on wound healing.

Takahashi concluded that when new vessels appeared in irradiated areas they had grown in from nonirradiated regions. We did not observe ingrowth of nonirradiated vessels unless a pathway was provided for them by the formation of a host cellular layer. Our finding that after irradiation new vessels continued to appear for 3 days may be explained by Takahashi's report that after irradiation canalization of newly formed vessels was slowed.

Evidence that there is some condition in an irradiated environment that indirectly affects tumor growth comes from reported studies of implants of tumor tissue placed on an irradiated bed [see review by Sugiura (*18*)]. These implants often failed to grow, or their growth was delayed. The inability of irradiated endothelium to produce new vessels, and the cellular reaction which is more apt to develop in an irradiated area than in a nonirradiated one, may contribute to the pecularities of growth of such implants.

SUMMARY AND CONCLUSIONS

The effect of irradiation on mammary tumor implants growing in transparent chambers in the skin of mice was studied *in vivo*. Irradiated tumor implants on a normal bed and normal implants on an irradiated bed were also observed.

After a dose of 2,000 or 3,000 r there was marked regression followed by regrowth. The slight opacity observed 1 day after irradiation was evidently caused by a direct action of irradiation on the tumor cells. An increased opacity during the 2d to 5th days may have been in part an indirect response attributable to a slow circulation. The tumor vessels narrowed progressively for approximately 1 week, clearly because the tumor tissue stopped growing; and when growth was resumed, the vessels enlarged again.

The growth rate of regrowth foci was at first the same as that of nonirradiated tumor tissue but since no new capillaries were produced the

vascular supply became inadequate and growth was slowed. The vessels within the regrowth foci broke down and the nonvascularized, relatively opaque tissue aroused a series of reactions resulting in the ingrowth of nonirradiated vessels into the irradiated zone. Although the chambers did not last long enough to allow observation of the vascularization of regrowth foci by nonirradiated vessels, it was indicated that when regrowth foci were penetrated by nonirradiated vessels normal growth would be resumed, since the growth rate was normal wherever irradiated tissue had an adequate vascular supply, that is, during the first few days of regrowth and in tissue regrowing after transplantation to a nonirradiated chamber.

That irradiated vessels could not produce new ones was demonstrated, not only by the vascular abnormalities of regrowth foci, but also by the inability of an irradiated bed to vascularize tumor implants or host-formed cellular layers.

REFERENCES

(*1*) ALGIRE, G. H., and LEGALLAIS, F. Y.: Recent developments in the transparent-chamber technique as adapted to the mouse. J. Nat. Cancer Inst. 10: 225–253 (1949).

(*2*) BARRETT, M. K., and MORGAN, W. C.: A maternal influence on the growth rate of a transplantable tumor in hybrid mice. J. Nat. Cancer Inst. 10: 81–88 (1949).

(*3*) BLUM, H. F.: Estimation of growth rates of tumors. J. Nat. Cancer Inst. 4: 21–24 (1943).

(*4*) ALGIRE, G. H., and CHALKLEY, H. W.: The vascular supply of mammary gland carcinomas. *In* A symposium on mammary tumors in mice. Am. Assoc. Adv. Sc. Publ. No. 22, 47–54 (1945).

(*5*) TAKAHASHI, T.: The action of radium upon the formation of blood capillaries and connective tissue. Brit. J. Radiol. 3: 439–445 (1930).

(*6*) CLARK, E. R., HITSCHLER, W. J., KIRBY-SMITH, H. T., REX, R. O., and SMITH, J. H.: General observations on the ingrowth of new blood vessels into standardized chambers in the rabbit's ear, and the subsequent changes in the newly grown vessels over a period of months. Anat. Rec. 50: 129–167 (1931).

(*7*) JOLLES, B., and KOLLER, P. C.: The role of connective tissue in the radiation reaction of tumors. Brit. J. Cancer 4: 77–89 (1950).

(*8*) WINDHOLZ, F.: Problems of acquired radioresistance of cancer: adaptation of tumor cells. Radiology 48: 398–404 (1947).

(*9*) LASNITZKI, I.: A quantitative analysis of the direct and indirect action of X radiation on malignant cells. Brit. J. Radiol. 20: 240–247 (1947).

(*10*) PULLINGER, B. D.: Causes of cell death in irradiated human tissue. J. Path. & Bact. 35: 527–540 (1932).

(*11*) DESJARDINS, A. V.: Causes of cell death in irradiated tissue. Am. J. Roent. & Radium Therapy 28: 398–401 (1932).

(*12*) WOOD, F. C.: The vascular changes produced by radium and X-rays. International Conference on Cancer, London, (1928).

(*13*) WARREN, S.: Mechanism of radiation effects against malignant tumors. J. A. M. A. 133: 462–463 (1947).

(*14*) MOTTRAM, J. C.: A factor of importance in the radiosensitivity of tumors. Brit. J. Radiol. 9: 606–614 (1936).

(*15*) PATERSON, R.: The Treatment of Malignant Disease by Radium and X-rays. London. Edward Arnold & Co. (1948).

(*16*) SEELIG, M. G., ECKERT, C. T., and COOPER, Z. K.: The relationship between vascularity and the reaction to radium of squamous epithelium. Am. J. Cancer 25: 585–591 (1935).

(*17*) GOLDFEDER, A.: Further studies on the relation between radiation effects, cell viability, and induced resistance to tumor growth; comparison of effects of roentgen rays on mammary tumors autogenous to inbred strains of mice (dba and C3H). Radiology 49: 724–731 (1947).

(*18*) SUGIURA, K.: Studies on radiosensitivity of mouse sarcoma 180 irradiated *in vivo* and *in vitro*. Radiology 29: 352–361 (1937).

922686—51——18

PLATE 68

FIGURE 1.—Experiment 33. Tumor just before irradiation. × 50.
FIGURE 2.—Experiment 33. Tumor 1 day after irradiation. × 50.

922686—51——19

PLATE 69

FIGURE 3.—Experiment 17. Tumor just before irradiation. × 50.

FIGURE 4.—Experiment 17. Tumor 3 days after irradiation. The vessels are conspicuously narrower. Note the area at the lower right edge that was vascularized after irradiation. Arrow is for orientation with the next figure. × 50.

3

4

PLATE 70

FIGURE 5.—Experiment 17. Tumor 21 days after irradiation. Regrowth has been apparent for 1 or 2 days in the area that became vascularized during the first 3 days after irradiation. Vessels are enlarged and sparse in the regrowing area. On the left is the regressing portion of the tumor. × 50.

FIGURE 6.—Experiment 17. Tumor 25 days after irradiation, 5 or 6 days after regrowth became apparent. × 50.

FIGURE 7.—Experiment 17. Tumor 32 days after irradiation, 12 or 13 days after regrowth became apparent. The opaque area to the right (see arrow) is caused by growth without vascularization. Since the focus was at the surface of the tumor the regrowing tissue spread between the mica and muscle. × 50.

922686—31——20

PLATE 71

FIGURE 8.—Experiment 34. Tumor 2 days after irradiation. × 50.

FIGURE 9.—Experiment 34. Tumor 7 days after irradiation. × 50.

FIGURE 10.—Experiment 34. Tumor 16 days after irradiation. Regrowth in the central portion of the tumor was detected on the 14th day. A second focus indicated by the arrow shows slight enlargement of vessels. × 50.

FIGURE 11.—Experiment 34. Tumor 19 days after irradiation. Many vessels in the first regrowth focus have disintegrated. In the second focus the space marked by a star can be followed in the next three figures. × 50.

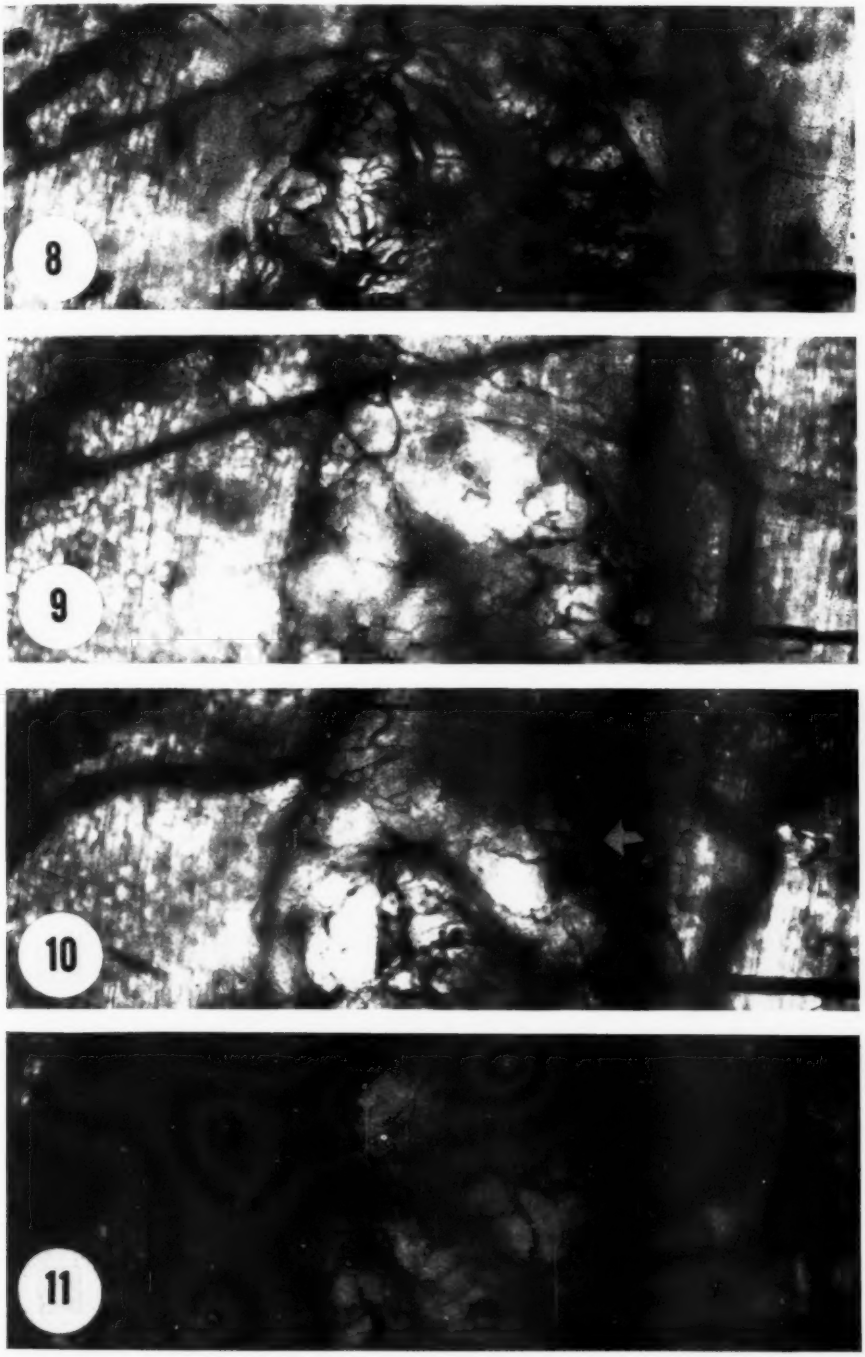
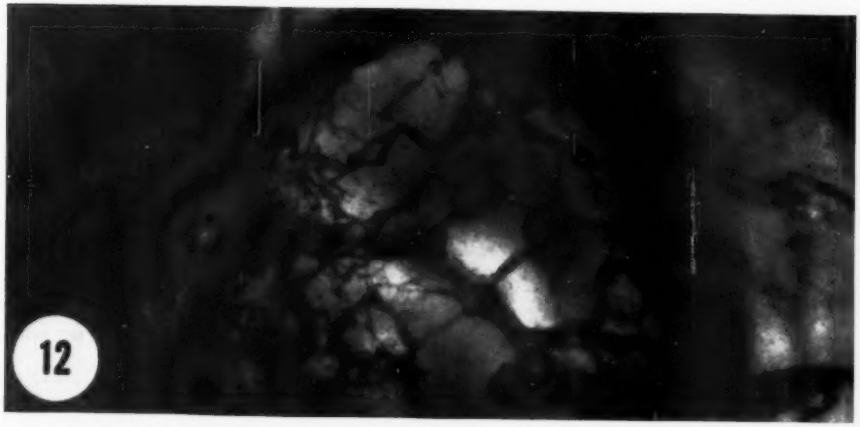

8
9
10
11

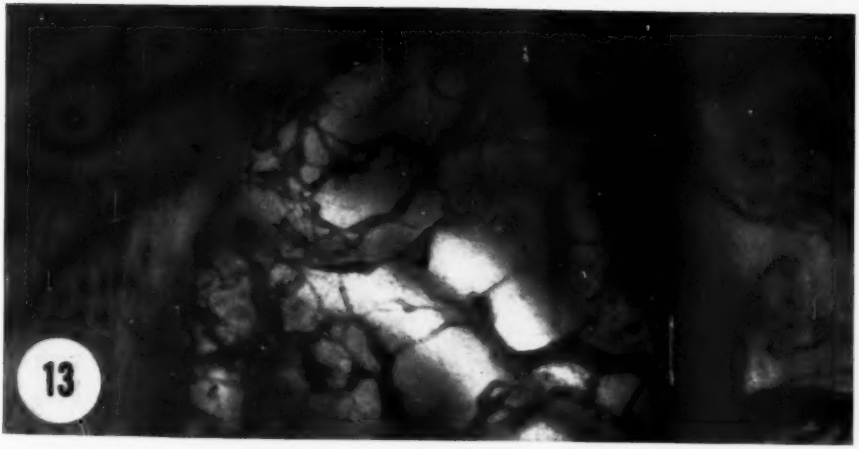
PLATE 72

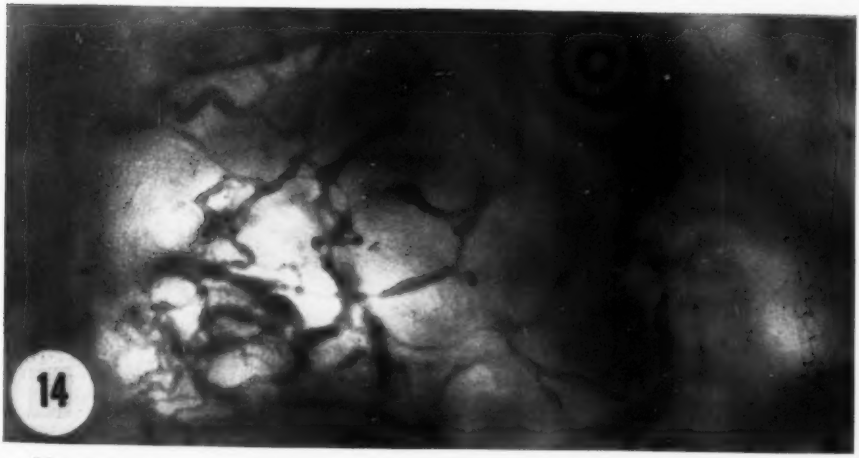
FIGURE 12.—Experiment 34. Tumor 22 days after irradiation. In the second regrowth focus the vessels are becoming larger and sparser and no new vessels have formed. × 50.

FIGURE 13.—Experiment 34. Tumor 23 days after irradiation. Vessels in the second focus are narrowing again. × 50.

FIGURE 14.—Experiment 34. Tumor 28 days after irradiation. The second focus has been apparent for 11 days. Vessels have fragmented and there is stasis in others. Opaque areas can be seen at points farthest from vessels. × 50.







922686—51——21

PLATE 73

FIGURE 15.—Experiment 10. Tumor 20 days after irradiation. Regrowth has not begun in the large tumor, a portion of which fills the right side of the figure. The small separate tumor on the left of the figure was vascularized during the first 3 days after irradiation and has been regrowing several days. × 50.

FIGURE 16.—Experiment 10. A photomicrograph 33 days after irradiation, about 16 days after regrowth began. The implants lie between the muscle layer of the skin and a very thick layer of host cellular reaction. The regressing tissue on the right is stained less deeply and fat cells can be seen embedded in it. Pale staining areas of necrosis can be seen in the regrowing tissue. Opaque areas had been visible for less than 11 days but more than 5 when the tumor was fixed. × 50.

15

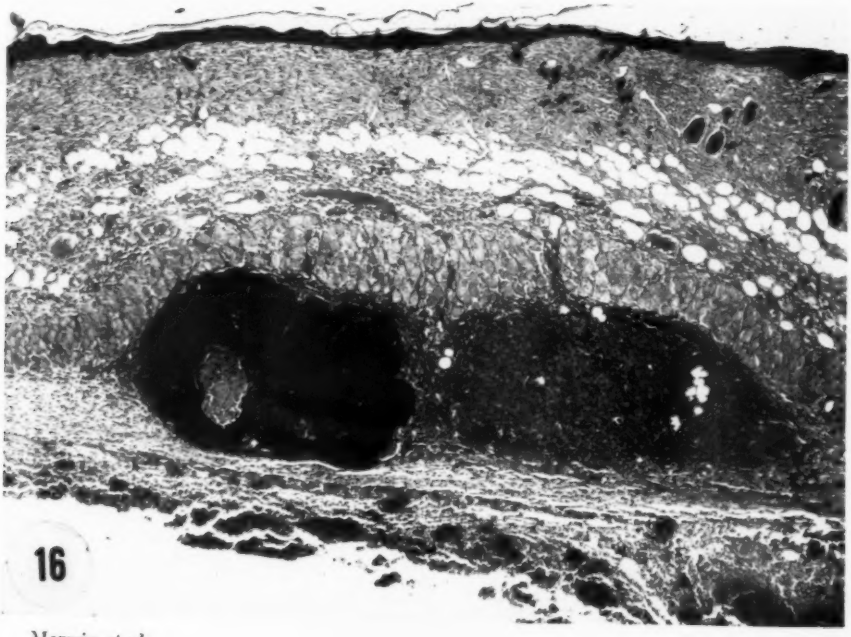

16

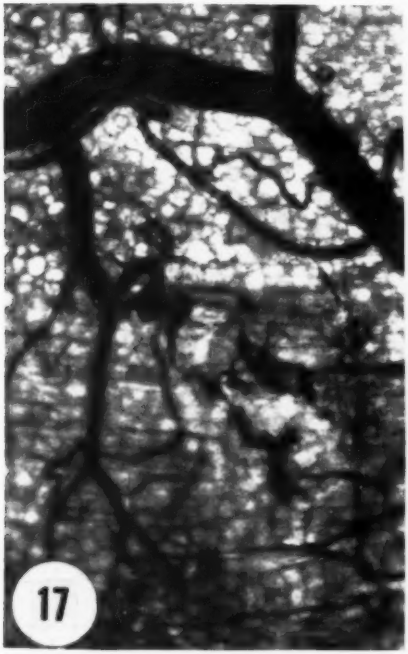
PLATE 74

FIGURE 17.—Experiment 47. Tumor 2 days after implantation of tumor tissue in an irradiated window. The blood vessels which do not look normal disintegrated by the next day. Note the hemorrhage at the right of the implant. Other vessels appeared in the succeeding days. × 50.

FIGURE 18.—Experiment 47. The tumor appears 7 days after implantation as a hazy area. The vessels within the tumor have enlarged. × 50.

FIGURE 19.—Experiment 47. Tumor 10 days after implantation. The vessels are still enlarging. × 50.

FIGURE 20.—Experiment 47. Tumor 17 days after implantation. New vessels have been formed and the old ones have become narrower. The tissue farthest from vessels is more opaque. × 50.


17

18

19

20

PRELIMINARY REPORT OF CONFERENCE ON GEOGRAPHICAL PATHOLOGY AND DEMOGRAPHY OF CANCER

HELD BY

COUNCIL FOR THE CO-ORDINATION OF INTERNATIONAL CONGRESSES OF MEDICAL SCIENCES

UNDER THE AUSPICES OF

WORLD HEALTH ORGANIZATION (WHO)

AND

UNITED NATIONS EDUCATIONAL, SCIENTIFIC, AND CULTURAL ORGANIZATION (UNESCO)

SESSIONS HELD AT

REGENT'S PARK COLLEGE, OXFORD, ENGLAND

JULY 29 TO AUGUST 4, 1950

922686—51——23

CONFERENCE ON GEOGRAPHICAL PATHOLOGY AND DEMOGRAPHY OF CANCER

PRELIMINARY REPORT BY J. CLEMMESEN[1][2]

The conference on the Geographical Pathology and Demography of Cancer was convened by the Council for the Co-ordination of International Congresses of Medical Sciences, (C. C. I. C. M. S.), thus giving effect to a recommendation of the International Cancer Research Commission.

The Conference held its sessions at Regent's Park College, Oxford, England, from July 29 to August 4, 1950, under the chairmanship of Professor J. Maisin. The scientific secretary during the preparatory stages of the conference and at its sessions was Dr. Johannes Clemmesen.

The following members were present:

J. Maisin, M. D., *Chairman*	President C. C. I. C. M. S., Professor of Pathology, University of Louvain, Belgium.
J. Clemmesen, M. D.	Scientific Secretary. Chief Pathologist. Director Cancer Registry, Copenhagen, Denmark.
J. F. Delafresnaye, M. D., M. R. C. P.	Executive Secretary, C. C. I. C. M. S., Unesco House, Paris, France.
P. F. Denoix, M. D.	Chirurgien des Hôpitaux de Paris, Chef des Services techniques et de la Section du Cancer, Institut National d'Hygiène, Paris, France.
R. Doll, M. D., M. R. C. P.	Member of Statistical Research Unit, Medical Research Council, London, England.
H. F. Dorn, Ph. D.	Chief, Biometrics Branch, National Institutes of Health, Bethesda, Maryland, U. S. A.
J. Engelbreth-Holm, M. D.	Professor, University Institute of Pathological Anatomy, Copenhagen, Denmark.
J. Gillman, D. Sc., M. B., B. ch.	Professor of Physiology, Director of the Joint Unit of the Council of Scientific and Industrial Research and of the University of Witwatersrand, Johannesburg, South Africa.
Sir Ernest Kennaway, M. D., F. R. S.	St. Bartholomew's Hospital, London, England.
V. R. Khanolkar, B. Sc., M. D., F. N. I.	Vice-President, Indian Academy of Sciences. Hon. Director of Laboratories, Tata Memorial Hospital, Bombay, India.
W. Kouwenaar, M. D.	Professor of Tropical Medicine. Instituut voor tropische Hygiene, Amsterdam, Holland.
A. Lascassagne, M. D.	Professeur au Collège de France, Directeur de l'Institut du Radium, Paris, France.
J. Mussini-Montpellier, M. D.	Chef des travaux d'anatomie pathologique de la Faculté de Médecine d'Alger. Chef de Service (Fichier central) du Centre algérien de lutte contre le cancer, Alger, Algeria.
Charles Oberling, M. D.	Professeur de carcinologie à la Faculté de Médecine de Paris, Paris, France.

[1] The complete report will appear in ACTA, Unio Internationalis contra Cancrum, Louvain, Belgium.

[2] Reprints may be obtained from the Editorial Office, National Cancer Institute, Bethesda 14, Maryland, U. S. A., or from Dr. J. C. Clemmesen, Director, The Danish Cancer Registry, Strandboulevarden 49, Copenhagen, Denmark.

922686—51——24

B. S. Platt, C. M. G., M. B. M. Sc. Ph.D.	Professor of Nutrition, University of London. Director, Human Nutrition Research Unit, Medical Research Council, England.
A. H. T. Robb-Smith, M. D., M. R. C. P.	Chief Pathologist, Radcliffe Infirmary. Nuffield Reader in Pathology, Oxford, England.
P. Stocks, C. M. G., M. D., F. R. C. P.	Chief Medical Statistician. General Register Office, London, England.
H. L. Stewart, M. D-------	Chief, Pathology Section, National Cancer Institute, Bethesda, Maryland, U. S. A.
A. Symeonidis, M. D., Ph. D.	Special Adviser, Geographical Pathology Unit. National Cancer Institute, Bethesda, Maryland, U. S. A.
Sutomo Tjokronegoro, M. D.	Professor of Pathology, University of Indonesia, Djakarta, Indonesia.
M. B. Visscher, M. D., Ph. D.	Professor and Head of Department of Physiology, University of Minnesota, Minneapolis, Minnesota, U. S. A.
J. M. May, M. D---------	Chairman of Commission on Medical Geography. American Geographical Society, New York, New York. (Was unable to attend but Dr. Dorn agreed to present his paper.)

The purpose of the Conference was to study the geographical variations in the incidence and behavior of cancer and to consider which problems among those revealed by its study could be subjected profitably to further investigation among the affected populations and to experimental study with the ultimate aim of assisting in the prevention of cancer in man.

The first task of the Conference was, therefore, to sift available information in order to determine what evidence there is of variations in the distribution of cancer in various parts of the world. The Conference soon realized that the limitations of such studies were due mainly to the lack of information on population statistics in many parts of the world, to the lack of facilities for the exchange of knowledge and local experience between investigators, and to the unsatisfactory presentation of statistical data in many publications, which renders comparisons difficult.

The Conference therefore deemed that it should consider the ways and means of improving conditions in this important branch of study.

It is the intention in the following report to set forth the recommendations adopted by the Conference and to give short abstracts of papers followed by discussion. The full report of the Conference will be published at a later date in the ACTA of the International Union against Cancer.

The subject matter of the recommendations falls under three headings:

A. Review of available information on the variations in the incidence and behavior of cancer and suggestion as to future work.

B. Guidance on the presentation of statistical studies of geographical and other variations in the incidence of cancer.

C. Advice on the methods for the provision of a mechanism by which studies in this field could be promoted, assisted, and made more fruitful by cooperation.

RECOMMENDATIONS

Review of present information and promotion of further study.—The Conference on Geographical Pathology and Demography recognizes the serious lack of information on population statistics and other data necessary for the computation of rates of cancer incidence in different regions. Despite these limitations there is evidence of variations in the distribution of cancer in various parts of the world.

There is presumptive evidence that:

(*a*) Primary cancer of the liver is relatively frequent in Africa and Indonesia. It also appears to be common in Greece, India, Malaya, and the Philippine Islands.

(*b*) Cancer of the cervix uteri is relatively infrequent in Jewish women.

(*c*) Cancer of the stomach is relatively infrequent in Javanese, African Negroes, and the indigenous people of French North Africa.

(*d*) Cancer of the base of the tongue is relatively frequent in certain communities in India.

(*e*) Cancer of the scalp is frequent in inhabitants of French North Africa, of the skin of the trunk in inhabitants of India, of the scalp and of the skin of the legs in African Negroes and Indonesians.

(*f*) Relatively large numbers of cases of cancer of the pancreas have been reported from Mulago Hospital, Kampala, Uganda.

(*g*) In some tropical countries (Indonesia, India, and Indochina) an unusual number of malignant tumors of cervical lymph nodes has been reported.

The Conference considered that the part played by race, nutrition, infections, and environment might be so important in carcinogenesis that special efforts should be made to include investigations of the interaction of these factors in the endemiological study of cancer. The Conference gave special consideration to the means by which advances could be made in regions where few or no meetings occur between groups of workers in fields of closely related interest. The following recommendations were therefore adopted:

RECOMMENDATION 1. The Conference on the Geographical Pathology and Demography of Cancer

Recommends

1.1 That facilities be provided for an exchange of knowledge and local experience between investigators working on similar programs in the endemiology of cancer.

1.2 That periodical meetings of such workers be arranged in places where such projects are being carried out, particularly among workers in adjacent areas.

1.3 That a study group devise methods and procure means for investigating the occurrence of cancer in populations for which statistics are not available, giving particular attention to the determination of age in the population.

1.4 That special studies be made on the interaction of race, nutrition, infection, and environment on the problems of carcinogenesis, taking full advantage of the work already done by the specialized agencies of the United Nations.

1.5 That two problems be undertaken and integrated with the work already in progress in the localities concerned: primary cancer of the liver; and cancer of the cervix uteri in Jewish women. It will be desirable to investigate simultaneously the problems of cancer of the breast.

Statistical studies of geographical and other variations in the incidence of cancer.—The Conference considered the methods available for statistical studies of geographical and other variations in the incidence of cancer, and adopted the following recommendation:

RECOMMENDATION 2. The Conference on the Geographical Pathology and Demography of Cancer, considering that it is desirable to obtain a high degree of comparability of results

Recommends

2.1 That all authors and editors of future publications on the frequency of malignant disease shall provide information on the following essential points, if possible separately for each site affected.

2.1.1. Total number of new cases in the area being studied.

2.1.2. Distribution of the total population and of patients in the area with respect to race, sex, and age.

2.1.3. Percentage of cases diagnosed in hospital.

2.1.4. Percentage of cases diagnosed by histological examination.

2.1.5. Percentage of cases verified by autopsy.

2.1.6. Description of any subdivision undertaken (social, occupational, geographical, *etc.*).

2.1.7. Statement on the type and location of hospitals from which statistics have been gathered.

This recommendation was adopted as the result of the following considerations:

It was realized that the increasing efficiency in the therapy of cancer tends to make mortality statistics increasingly inadequate as a measure of the real incidence of malignant disease. Studies of the mortality from cancer may, however, still be useful for certain purposes such as studies of cancer of specific sites. It was especially pointed out that if such studies are made for rural and urban populations, appropriate adjustments should be made for deaths of nonresidents.

It was agreed that, ideally, studies of cancer incidence in a given area should contain full information concerning the total number of new cases of malignant disease, diagnosed within the period under consideration, as far as possible for each site separately. If special studies are carried out for a single site, information as to the total number of cases of all sites within the material treated should be given whenever possible.

In addition to full subdivision with respect to primary site, a complete description should be given of the age, preferably in quinquennial groups, sex, and racial composition of the general population from which the cancer cases have arisen.

Cases with a diagnosis of "possible" or "probable" cancer may be shown by a total for each site, but should not be included in the statistical treatment. Although diagnoses of malignancy confirmed by histological examination (biopsy or autopsy) are the most reliable, statistical data for such cases alone usually will be unrepresentative of all cases of cancer developing in a population. Special analysis of histologically confirmed cases may be useful in evaluating the reliability of the conclusions drawn from the total data, although the value of this test will vary with the percentage of cases verified histologically. Whenever possible, the percentage of cases histologically confirmed should be stated for each site separately.

When studies are made of the occurrence of cancer in various social classes, or other groupings, the criteria of such subdivisions should be clearly stated.

Studies of cancer incidence in urban and rural areas should give the basis for this classification as well as information concerning such factors as density of population, character of industries in the area concerned, and if the population is living on the products of the area, the type of cultivation, *etc.*

When conclusions are drawn from hospital cases alone full information should be given concerning the extent of the area served and the type of case admitted to the hospital.

Publications should contain tables giving the fundamental figures mentioned above for the entire population as well as for the cancer patients, so that other workers will be able to make comparisons or analyses apart from those in the original publication. Likewise, all formulas used in the computation of results should be given in full, so that readers can study both methods and results obtained.

It is desirable also that results should be illustrated by diagrams or graphs, so that readers specialized in other branches of cancer research may form an opinion of the results obtained without a detailed study of tables and figures. The omission of these details in order to reduce the expense of printing will decrease the value of the paper concerned.

Promotion, assistance, and cooperation in the field of the endemiology of cancer.

RECOMMENDATION 3. The Conference on Geographical Pathology and Demography of Cancer

Recommends

3.1 That the C. C. I. C. M. S. establish a Study Section for the endemiology of cancer with the functions, general methods of operation, and for the purposes described in the following outline:

3.1.1. This Conference on the Geographical Pathology and Demography of Cancer recognizes the desirability of providing a mechanism by which the studies of investi-

gators in the field of the endemiology of cancer can be promoted, assisted and made more fruitful by cooperation. It is also aware of the fact that there should not be unnecessary duplication of organization and effort among international bodies concerned with the endemiology of cancer. The Conference therefore recommends that with a view to implementing the recommendations of the International Cancer Research Commission at their meetings in 1949 and 1950 there be established, under the auspices of the C. C. I. C. M. S., a Study Section on the endemiology of cancer, consisting initially of not more than fifteen members, drawn from among active investigators in the field of endemiological studies in cancer, and appointed by the C. C. I. C. M. S. after consultation with the International Cancer Research Commission. The members of the Study Section should be appointed for terms of not more than 3 years but should be eligible for immediate reappointment. The terms should be initially so arranged that a constant fraction expires each year. It would be hoped that in this way the Study Section so established might serve as a coordinating mechanism for all work in the endemiology of cancer.

The field of the endemiology of cancer will be broadly defined as comprising knowledge concerning variations in the distribution and behavior of cancer among various ethnological groups in different localities in relation to any relevant local factors. Such knowledge is sought in order to elucidate the causes of cancer or to suggest experimental studies which may do so, with the ultimate aim of assisting in the prevention of cancer in man.

3.2. The function of the Study Section on the endemiology of cancer should be:

3.2.1. To serve as a point of contact and communication between investigators in its field of interest in different parts of the world, implementing this function by such means as the collection and distribution of lists of projects in the endemiology of cancer under active investigation in particular laboratories, so that coordinated attack on specific problems may become easier.

3.2.2. To invite the cooperation of cancer investigators to serve as correspondents, to promote the establishment of centers of information in the various regions and countries of the world, to encourage the formation of national and regional study sections and assist in the collection of scientific publications on the endemiology of cancer.

3.2.3. To encourage and facilitate cooperation on specific scientific problems in endemiological aspects of cancer biology by assisting in the procurement of grants-in-aid for research upon such problems after the Study Section has considered the merits of particular projects, both as to scientific importance and as to practical feasibility.

3.2.4. To call together at appropriate times small groups of experts concerned with particular problems in the endemiology of cancer for conferences to collect, compare, and evaluate their observations, and to plan future investigations, especially when the latter will require international collaboration. The Study Section should itself meet at appropriate intervals with such conferences.

3.2.5. To promote the publication of all important information on the endemiology of cancer in journals devoted to cancer research.

3.3. The proposed Study Section on the endemiology of cancer of the C. C. I. C. M. S. should seek the assistance of the latter organization in providing secretarial facilities. The optimum arrangement would be for the central office of the Study Section to be situated in the office of the C. C. I. C. M. S., and for the Executive Secretary of the latter to serve in the same capacity for the Study Section.

3.4. It is recommended that C. C. I. C. M. S. provide funds for secretarial expenses and for travel costs of the occasional conferences necessitated by the functions outlined previously. It is expected that funds for specific research projects can be obtained from other sources, but it is recommended that the parent agencies of C. C. I. C. M. S. be encouraged to assist also in specific research activities.

J. Delafresnaye, *executive secretary*

922686—51——25

SECRETARIAL ADDRESS

By J. Clemmesen

It follows from the very name of this conference that it will deal with two aspects of what has so far been termed the epidemiology or geographical pathology of cancer or geographical cancer research. It is our aim to study variations in the incidence and behavior of cancer. Furthermore, it is intended to examine which studies of this kind may inspire experimental investigations, and, conversely, which experimental evidence might instigate examinations in the field of geographical pathology and demography of cancer. We shall for the time being have to exclude the field of industrial cancer, which certainly belongs in our field of interest but would comprise too many and too special problems for this meeting.

Geographical pathology in its stricter sense has in the past given much valuable information, not the least of which has come from less sophisticated areas in all parts of the world. Within the field of oncology, observations such as those on kangri cancer, or skin cancer among fishermen, have been fundamental to our ideas on the etiology of human cancer, and it is not unlikely that this line may be continued.

Demographical—or statistical—studies in cancer demand for their establishment a basis of statistical information on the normal population, available only in civilized countries with highly developed statistical and medical services, which naturally inclines to restrict the areas where studies are feasible.

Such studies have already yielded interesting information, but are not known as widely as results from geographical studies in pathology, probably because of some reluctance of the medical world to enter the statistical field in connection with rather scanty publicity in the cancer journals.

It would indeed be disastrous if these two branches of the same discipline should be regarded separately. Results from less-developed areas may serve as an inspiration to statistical studies in the highly civilized countries, and the latter may in their turn serve as a check on observations made on less-solid foundations, and may even sometimes indicate which problems may profitably be attacked with more direct means. Even imperfect statistics may sometimes prove a valuable inspiration to further research.

Statistical studies on the incidence of cancer are on the whole very uncomplicated, compared with the complexity of statistical studies involved in experimental work. They are, however, valuable in simplifying various problems facing us in our research, and should to a far larger extent be known by workers in other fields of cancer research, and thus contribute to a more practical outlook.

It is for practical reasons that this conference has been restricted to a limited number of workers interested in the field, and we hope in the future to benefit from the interest of those highly qualified workers who have been prevented from attending. It was intended in planning the work to cover as many geographical areas and as many different branches of cancer research as possible. It is the wish of the C. C. I. C. M .S.—The Council for Coordination of International Congresses of Medical Sciences—that practical results should be obtained, and we have informed this body and the International Cancer Research Commission that we would endeavor to outline:

1. Measures for forwarding publications in our field.
2. The setting up of a committee or other body with the aim of forwarding research in the geographical pathology and demography of cancer.
3. Other methods by which contact could be made between research workers in this field from various countries.
4. Subjects which particularly might deserve attention of workers in the near future.

Finally, I should find it desirable to stress the limitations of the fields in which we are working.

As to the demographical line, even the best of research could not provide us with a better material than the material provided by the English Registrar-General on the

increased risk of esophageal cancer among occupations exposed to alcohol. Nor will geographical pathology for a long period hence be able to produce better evidence than Dr. Khanolkar's material on oral cancer in India.

But we have to realize that even with such material at hand we are incapable of producing direct proofs. It is in the nature of such studies that they can only exclude some possibilities and suggest others, but the final proof has to be produced by other means—most often by experimental or clinical work. As a parallel it may be mentioned that it was through statistical studies that the connection between general paralysis and syphilis was first suggested, but the proof had to be produced by serological and bacteriological studies.

It is therefore fortunate that studies like those mentioned can be exploited for the inspiration of experimental work in the same way in which experimental medicine takes inspiration from clinical observations.

It was originally the discovery of cancer in chimney sweeps, in principle attained through mass observation by Percival Pott, which finally led to the discovery of the carcinogenic hydrocarbons by Cook, Hieger, Hewett, and Kennaway, but this is unfortunately an exception.

As an example of what might be more within reach, I should mention that workers at the University of Cincinnati have taken inspiration from observations on the variations of cancer incidence with climate, and provided experimental evidence on mice with regard to the effects of changes of temperature.

It is only through such combination of observations on human cancer, and experimental research and other biological studies that we can achieve our aims. Without such contact with other branches of cancer research our work—and perhaps not only ours—will be a failure.

SYNOPSIS OF PAPERS AND DISCUSSIONS

Variations of Cancer Mortality With Environmental Factors. PERCY STOCKS, *General Register Office, London, England.*

Abstract. Dr. Stocks presented for discussion preliminary results from extensive studies on mortality from cancer of various sites in large towns in England and Wales, excluding London, for the period 1921 to 1939, and also with regard to social classes. He dealt with cancer of the following sites: stomach, rectum, prostate, bladder, uterus, breast, and lung.

Since these results were preliminary, the discussion will be regarded as confidential so far as it refers to the results of Dr. Stocks.

Discussion

DR. CLEMMESEN: The interesting results of Dr. Stocks draw attention to one of the most outstanding differences in cancer mortality between England, the United States, and most other countries, namely the difference in mortality from gastric cancer.

The conception of this difference has originally been based on calculations of the percent of gastric cancer among all cancer deaths, which may allow a rough estimate, but pays no attention to the important influence of differences in the average age of the population. Incidentally, previous tables on this subject have most often been based on material from different years for the various countries.

The differences in mortality from gastric cancer are not unimportant, since studies from the later years have demonstrated that differences in mortality from cancers of inaccessible sites between Denmark, England, and Switzerland are largely due to gastric cancer as far as males are concerned, and wholly due to this cancer in the case of the females (Clemmesen and Busk, 1947–49).

A comparison of age-distribution curves will show that the differences in mortality from gastric cancer between England on one side, and Denmark and Switzerland on the other, are not due to any difference in the age distribution of the population. However, the difference may still not be real, but may be due to differences in clinical facilities and clinical traditions on nomenclature.

The author demonstrated a diagram showing the percentage of cases of gastric cancer among deaths from Danish rural and urban areas, as well as the capital, for three subsequent periods of years during which medical facilities were steadily improved. It was seen that in periods and in areas with good medical facilities the percentage of gastric cancer is decreasing. It has been verified by other computations that this decrease is not due to detection of more cases of cancer in sites other than the stomach.

It would seem that these observations deserve attention, together with the fact that gastric cancer in England seems more frequent in the towns than in the rural areas. It seems that it would be worth while to carry out comparisons between the ways diagnosis is established in England and Denmark, and in towns and rural areas of both countries. Perhaps an exchange of research workers might facilitate such a comparative study.

DR. SYMEONIDIS: I think it would be valuable if we could have a statement made as to how far mortality statistics will carry, compared with morbidity statistics.

DR. MAISIN: It would also be a result of practical importance if we could examine the various parts played by statistics on the basis of examinations made by clinical methods, by biopsy, and on autopsy material. It would be of particular importance to know the present attitude of investigators in the statistical field toward the necessity of histological confirmation of diagnosis.

DR. DENOIX: With regard to the last item, I believe it can be said at the moment that material restricted to cases histologically verified, would not be representative of the total material to the extent necessary for statistical studies.

DR. CLEMMESEN: Ideal statistics would comprise all cases occurring within a given area, and within a certain period, and only these cases. I think that it would be safe to adopt the principles applied by the Danish Cancer Registry: The total number of cases is stated for each site separately, but followed by the numbers for cases treated or diagnosed in hospital. For these cases the percentage of histological confirmations and of autopsies performed is stated. Such percentages may, however, vary for many reasons. Skin cancer would thus be histologically verified fairly often, because of its accessibility, while a diagnosis such as sigmoid cancer would mostly be used when autopsy had made accurate localization of the tumor possible. Such autopsy material would naturally include many cases histologically confirmed, although this is no indication of high reliability of the clinical diagnosis.

DR. SYMEONIDIS: I would like to ask to what extent clinical material can be used when it is collected from institutions with good diagnostic facilities.

DR. CLEMMESEN: Unfortunately the applicability of such materials has proved very limited for purposes of a character more general than therapeutic statistics. Knowledge of the normal population from which the cases have been drawn is essential, as soon as we wish to draw any conclusions as to the incidence of cancer. And the percentage of cases admitted in hospital may vary with the site of the tumor, as well as with the age and sex of the patient.

In the following discussion brief remarks were made by Drs. Visscher, Gillman, Lacassagne, and others.

The chairman suggested that Drs. Clemmesen, Dorn, and Oberling work out a draft for a recommendation on the statistical requirements for publication on statistical studies of geographical and other variations in cancer incidence. This recommendation, which was adopted in a later meeting, will be found in the beginning of the present report.

References

CLEMMESEN, J., and BUSK, T.: Cancer mortality among males and females in Denmark, England, and Switzerland. Cancer Research 7: 281–285, 1947.

———: Cancers of accessible and inaccessible sites. Cancer Research 8: 129–134, 1948.

———: Mortality of accessible and inaccessible cancers in Danish towns and rural areas. Cancer Research 9: 411–414, 1949.

STOCKS, P.: Cancer and goiter. Biometrika 15: 364–401, 1924.
———: Some further notes on cancer and goitre distributions. Biometrika 17: 159–165, 1925.
———: On the relation between the prevalence of thyroid enlargement in children and mortality from cancer and other diseases. Ann. Eugenics 2: 395–404, 1927.
———: On the evidence for a regional distribution of cancer in England and Wales. Report of the International Conference on Cancer. British Empire Cancer Campaign 508–519, 1928.
———: The distribution of cancer and tuberculosis mortality in England and Wales. Ann. Eugenics 4: 237–280, 1931.
———: A co-operation study of the habits, home life, dietary and family histories of 450 cancer patients and of an equal number of control patients. Ann. Eugenics 5:237–280, 1931.
———: The frequency of cancer deaths in the same house and in neighboring houses. J. Hyg. 35: 46–63, 1935.
———: Distribution in England and Wales of cancer of various organs. 13th, 14th, and 16th Annual Reports of British Empire Cancer Campaign 239–280, 1936; 198–223, 1937; and 308–343, 1939.
———: Regional and local differences in cancer death rates. Studies on Medical and Population Subjects, No. 1. General Register Office, London, 1947.
———: Cancer Statistics. *In:* Chamber's Encyclopedia. 1950.
———: Cancer of the stomach in the large towns of England and Wales. Brit. J. Cancer 4:147–157, 1950.

Variations in Cancer Incidence in the United States. HAROLD F. DORN, *Biometrics Branch, National Institutes of Health, Bethesda, Maryland, U. S. A.*

Abstract. This paper included a review of the methods applied in various parts of the United States for collecting information on morbidity and mortality from cancer.

Cancer-morbidity rates for most parts of the body other than genital organs and breast are higher for males than for females.

The incidence rate for all forms of cancer is nearly 50 percent higher among both white and negro persons living in the South than among those living in the North. This is due very largely to the higher incidence of cancer of the skin, and of the buccal cavity, especially the lip, among persons living in the South. Although skin cancer is relatively rare among Negroes, those living in the North and in the South show the same geographic difference as do white persons.

Negroes are more likely to have cancer of the genital organs. Nearly 70 percent of primary cancer in Negro females originates in the breast and genital organs. The incidence of cancer of the uterus among Negro females exceeds that among white females by about 60 percent. On the other hand, cancer of the breast and ovaries is more frequent among white females.

Discussion

DR. VISSCHER: Would it be possible to examine simultaneously the occurrence of cancer and other diseases, in order to establish—if possible—any relationship between such diseases and cancer?

DR. DORN: It would. It would mainly be an economical question to undertake such investigations.

DR. CLEMMESEN: The great value of such studies has been made clear by Dr. Helen O. Curth in recent studies on acanthosis nigricans and its relation to cancer. Studies on somatic types in relation to cancer and other diseases are in progress in Denmark.

DR. SYMEONIDIS: An inexpensive way to study the interrelation between cancer and other diseases would be the comparison of pathological material from different areas. Histological studies of liver tissue from Africa, India, Indonesia, and Greece, where primary cancer of the liver is relatively frequent, may, for instance, give in-

formation on other pathological conditions preceding the malignancy, and perhaps determine its development.

DR. DENOIX: I would like to emphasize the great value of the technique described by Dr. Dorn, which I have studied on the spot. It has, for the first time, made it possible to know the real frequency of cancer in a known population. In France, we have, so far, only used inquiry on a geographical basis in a way analogous to that of Dr. Dorn.

DR. SYMEONIDIS: What is the proportion of histological examinations in the United States?

DR. DORN: About two-thirds. A single figure would, however, be misleading, because there is considerable variation with the site. If, for instance, we exclude skin cancer, our percentage of histological examinations would rise.

DR. CLEMMESEN: In Denmark the same procedure would have the opposite effect. Incidentally, the percentage of histological examination is just one expression of the efficiency of diagnosis.

DR. DENOIX: Still I am impressed by the high figures from the United States, which indicate a high reliability of the material. I think valuable information may be obtained through comparative studies on American and African Negroes.

References

CURTH, H. O.: Cancer associated with acanthosis nigricans. Arch. Surg. 47: 517–552, 1943.

DORN, H. F.: Incidence and prevalence of cancer of the lung. Pub. Health Rep. 58: 1265–1271, 1943.

———: Illness from cancer in the United States. Pub. Health Rep. 59: 33, 65, & 97, 1944.

———: Morbidity from cancer. J. Washington Acad. Sc. 39: 117–125, 1949.

On Cancer Incidence in Denmark and Other Countries. JOHANNES CLEMMESEN, *The Danish Cancer Registry under the National Anti-Cancer League, Copenhagen, Denmark.*

Abstract. Since its opening in 1942 the Danish Cancer Registry has devoted a considerable part of its work to *genetical studies*, largely in collaboration with the University Institute for Human Genetics. A recent review will be found in the British Journal of Cancer.

The *organization* of the registry and its *incidence figures* with detailed distribution on age for all important sites have been published in Acta Radiologica. The registry deals with all cancer cases in Denmark. Hospital cases, about 80 percent, are all notified directly, and cases from general practice are collected from death certificates.

Studies on the mortality from cancer in various groups of Danish *occupations* for 1935 to 1939 have on the whole been in conformity with earlier English observations.

Thus esophageal cancer caused more deaths among persons employed in hotels, restaurants, *etc.*, and among commercial travelers, than in other occupations. A comparison with England and Switzerland showed an increased rate of male mortality for esophageal cancer in the latter country.

Gastric cancer caused fewer deaths than expected among the group "immaterial activities," while deaths from intestinal cancer were more frequent here than expected. In Denmark as in England it was difficult to judge whether these differences were caused by a better differential diagnosis or were of a more genuine nature.

Further comparative studies on cancer mortality in Denmark, England, and Switzerland have been described in an earlier part of this conference.

For inaccessible cancers, mortality figures showed no differences inside Denmark. But accessible cancers in women showed an excess for the capital, Copenhagen.

Studies on the incidence figures from the Registry showed that this could be ascribed to mammary and uterine cervical cancers and to a lesser extent to cancer of the uterine body.

Closer studies of subdivisions of the Copenhagen area disclosed that cervical cancer is less frequent in wealthy quarters, where house rent is high, and that a gradual increase attends the decrease of rent in other quarters. Rural areas showed the same low values as the wealthy quarters, which seems to exclude general hygiene as a decisive factor. Presumably the well-known correlation between the frequency of births and the incidence of cervical cancer is a better clue, if we assume that it is not the number of births, but the number of pregnancies, including abortions, through which social conditions influence the frequency of this cancer.

Breast cancer showed the opposite correlation to social status, although less pronounced, while cancer of the uterine body seemed evenly distributed throughout the city.

Special studies on breast cancer showed 111 cases located in the left breast for every 100 in the right. It has been demonstrated by Busk that the sisters and mothers of patients with cancer of the breast will tend to develop an eventual cancer at the same site as their suffering relative. This observation has later been confirmed by Penrose, *et al.*

At the climacteric age, 48 to 52, there is a decrease in the incidence of breast cancer.

In order to call attention to the necessity of an international approach to the problems of cancer statistics, the Danish Cancer Registry in September 1946 held an inter-European conference on the subject. A resolution was passed and delivered to the Interim Commission for the WHO (Schinz 1946).

Discussion

SIR ERNEST KENNAWAY: I would like to emphasize the great value of the material now being collected in Denmark by Dr. Clemmesen. It is of great importance that statistical studies on cancer should comprise a whole country, as in Denmark, where the medical data are of a high standard and the population at risk is known in detail. Denmark is ahead of all other countries in this respect.

MME. MUSSINI-MONTPELLIER: With regard to the Danish observations on breast cancer, and the irregularity of the age-distribution curve at the climacteric age, I would like to point out that in our studies on the Moslems in North Africa we have found no such irregularity at the menopause. However, the Europeans in North Africa show an irregularity similar to that found in the Danish investigations.

DR. OBERLING: I would recommend that an accurate estimation of the age for puberty and menopause be carried out for the populations in question.

DR. GILLMAN: This would be particularly useful with regard to the African population. There is suggestive evidence that puberty occurs relatively late in Africa, and the menopause rather early.

MME. MUSSINI-MONTPELLIER: I can confirm that for North Africa.

DR. PLATT: I think it would be appropriate to warn against the conception of a high or particularly intense metabolic activity in such a population with a late puberty and an early aging. There is evidence suggesting that it may be the question of a low and pathological metabolism, which nevertheless is followed by changes in the age at both puberty and menopause.

DR. CLEMMESEN: As soon as the irregularities in question were found, we realized the desirability of an accurate estimation of the average age at menopause for Danish women. We know that these ages differ for various countries, but their assessment is more complicated than one would expect. We have an earlier publication on the menopause age in Danish women by Clausager-Madsen and Ytting (1942), who found the average age for the menopause among 302 women in the capital to be 47.8 years and for 176 women from the country to be 48.1 years. One difficulty not generally realized is that women prefer unconsciously to give their age at the menopause in round figures, ending in 0 or 5, and they also prefer equal numbers to the unequal. Another source of error—which does not apply to our population—is that when the age of the normal population is less accurately stated, the irregularity of the curve may disappear.

DR. OBERLING: Would it not be an explanation of the irregularity that we are dealing with two different kinds of breast cancer?

DR. CLEMMESEN: That is in fact my opinion. The curves might for instance represent two types of cancer, sensitive to hormones to a different extent. We know already that some types of breast cancer, or their metastases, will react to hormonal therapy while others do not. Similarly, there is a less favorable reaction to radiological therapy at the age in question. It is also an interesting fact that the first decline of the breast-cancer curve coincides in age with the downward turn of the curve for cancer of the uterine cervix, the only cancer which shows a tendency to decrease in frequency with age.

DR. DENOIX: Age-distribution curves for cancer cases treated in French hospitals show a tendency to decline for the oldest age classes. How should this be explained?

DR. CLEMMESEN: Similar observations have been made on the curves for Danish hospital cases; for instance, patients over the age of 70 with breast cancer. However, the total for all cases shows an increase, the explanation apparently being that doctors refrain from sending the very old patients to hospitals. It is true that the total figures for rural areas show a similar decrease for the very old, but this is not the case for the capital, with its medical facilities; so that we think it unjustified to accept such a decrease in the old age classes as real.

References

BUSK, T., and CLEMMESEN, J.: The frequencies of left- and right-sided breast cancer. Brit. J. Cancer 1: 345–351, 1947.

CLAUSAGER-MADSEN, L., and YTTING, H.: Undersoegelser over Menarchens og Menopausens Indtraaeden i By- og Landbefolkningen. Nordisk Medicin 16: 3677–3681, 1942.

CLEMMESEN, J.: Cancer and Occupation in Denmark 1935-39. Copenhagen, Nyt. Nord. Forlag. 1941, pp. 75.

———: Carcinoma of the breast; results from statistical research. Brit. J. Radiol. 21: 583–590, 1948.

———: The status of genetical studies in human cancer. Brit. J. Cancer 3: 474–484, 1949.

———: Essentials of demographical studies in cancer. Brit. J. Cancer 4: 1–5, 1950.

CLEMMESEN, J., and BUSK, T.: I. Cancer mortality among males and females in Denmark, England, and Switzerland. Cancer Research 7: 281–285, 1947.

———: II. Danish towns and rural areas. Cancer Research 7: 286–289, 1947.

———: III. Cancers of accessible and inaccessible sites. Cancer Research 8: 129–134, 1948.

———: IV. Mortality of accessible and inaccessible cancers in Danish towns and rural areas. Cancer Research 9: 411–414, 1949.

———: The incidence of malignant diseases in Denmark 1942–44. Acta radiol. 29: 322–330, 1948; 30: 9–16, 1948.

CLEMMESEN, J., BUSK, T., and NIELSEN, A.: Age distribution figures for malignant diseases in Denmark 1942–44. Acta radiol. 31: 51–59, 1949.

PENROSE, L. S., MACKENZIE, H. J., and KARN, M. N.: A genetical study of human mammary cancer. Brit. J. Cancer 2: 168, 1948, and Ann. Eugenics 14: 234, 1948.

SCHINZ, H. R.: International Cancer Conference Sept. 1946 in Copenhagen. Schweiz. med. Wchnschr. 76: 1194–1196, 1946.

On the Etiology of Lung Cancer. RICHARD DOLL, *Statistical Research Unit, Medical Research Council, London, England.*

Abstract. A clinical investigation has been undertaken to assess the relationship between smoking and cancer of the lung. A close relationship has been found. It is, therefore, tempting to conclude that the increased number of deaths attributed to cancer of the lung is the result of the increased consumption of tobacco, but, in England,

the rate of increase of deaths attributed to cancer of the lung has been disproportionately rapid.

An attempt will be made to see how far the observed difference in incidence in men and women and in urban and rural inhabitants can be explained by different habits of tobacco consumption.

Discussion

DR. LACASSAGNE: In evaluating the part played by tobacco smoke in the etiology of lung cancer the number of cigarettes smoked is insufficient if no statement is made as to conditions under which they are smoked. Ten cigarettes a day may have a very different influence on the respiratory passages whether they are smoked in the open, or in a confined space. A smoker spending several hours a day in badly ventilated places like students' rooms, restaurants, workmen's quarters, offices, or railway compartments, *etc.*, will continuously inhale air filled with tobacco smoke. From this point of view the distinction between smokers and nonsmokers will in some cases be illusory. A nonsmoker forced to work in an office with heavily smoking colleagues may suffer an attack on his respiratory passages of a higher severity than a smoker employed in an open-air occupation.

DR. DOLL: Inhalation without smoking may not be injurious as the smoke is usually filtered through the nose, and thus is less likely to reach the bronchi.

DR. CLEMMESEN: It has been suggested by L. Cobbett in 1917 that the tubercle bacillus is transferred to the lung by the process of swallowing, which should send sputum containing the bacilli into the lung. The same mechanism might perhaps be taken into consideration for carcinogenic hydrocarbons too.

DR. DOLL: Our evidence indicates that tobacco chewing is negatively correlated with cancer of the lung. This is not surprising, as in England the majority of tobacco-chewers are people who cannot smoke because of the nature of their work.

MME. MUSSINI-MONTPELLIER: In North Africa women do not smoke, and we do not see any cases of lung cancer among them. Men do smoke, and they show a small number of lung cancers.

DR. CLEMMESEN: Mortality from lung cancer among males was nearly tripled from 1936 to 1945, for Copenhagen. The increase of figures for other parts of the country was far less pronounced, and the figures for females nearly constant. It would seem unlikely that this tripling, if real in nature, should not be reflected among the patients who were referred to the Central Tuberculosis Station for Copenhagen as suffering from chest trouble of unknown origin, but this was not the case.

Such an increase may be to a large extent due to improvements in diagnostic measures and in therapy against complicating pneumonias, which in earlier years may have been mistaken as primary cause of death for patients with a minute cancer in the lung. In this connection it may be of interest that the first lobectomy in Copenhagen was performed in 1939, and bronchoscopy was introduced in 1940, since the rise in mortality for lung cancer in Copenhagen is particularly pronounced between 1939 and 1942. A genuine rise in the incidence of lung cancer may, of course, exist but, so far, the rise in mortality may be explained as apparent. It is worth while to notice that the studies on cancer in areas of different house rent in Copenhagen tended to show a higher incidence of lung cancer among the poorer than among the wealthier classes.

Dr. Percy Stocks has demonstrated a parallelism between mortality from cancer of the lung and pollution of the atmosphere in English towns. I wonder if Dr. Dorn could inform us on American experience in that field.

DR. DORN: A comparison was made between Pittsburgh in the North and Birmingham in the South. Both are great steel centers and they show incidence figures similar to those of other towns.

DR. DOLL: If it is accepted that carcinoma of the lung is largely attributed to smoking, there still remains a residue of cases in nonsmokers that might be produced by atmospheric pollution in towns. It should be possible to determine the importance of this by comparing the incidence in nonsmokers in town and country. However, as Stocks has shown, smoking is more common in towns than in the country, and the

observation of a greater prevalence in the great conurbations may be associated with heavier smoking habits. Differences in smoking habits may thus account for the incidence in men in Greater London being 1.3 times the incidence in rural districts, and in women 1.5 times. The observed incidence in 1948 for men in Greater London was 1.9 times that of the rural districts, and for women, 2.0 times.

DR. SYMEONIDIS: It would be interesting to study lung cancer in Greece, where tobacco is produced within the country itself, and smoked almost exclusively as cigarettes. There seems to be some suggestive evidence that an increase of lung cancer has taken place but I know of no conclusive evidence.

SIR ERNEST KENNAWAY: What kinds of tobacco are smoked in Greece and in Denmark?

DR. ENGELBRETH-HOLM: In Denmark: English or American Virginia tobacco.

SIR ERNEST KENNAWAY: What do you mean by English tobacco?

DR. ENGELBRETH-HOLM: The kinds usually smoked in this country.

DR. SYMEONIDIS: In Greece the tobacco is so-called Turkish tobacco but it is grown in Greece. It is said that lung cancer is still rare in Turkey.

DR. OBERLING: I may add that cigarettes are smoked only by the wealthy in Turkey.

SIR ERNEST KENNAWAY: May the spraying of tobacco plants with special chemicals not have some relationship to the question of the carcinogenic properties of tobacco?

DR. DOLL: There is some evidence from death certificates that tobacco workers and tobacconists have an increased incidence of cancer of the lung. The increase is, however, not great, and has become less marked in recent years. The figures have been analyzed by Sir Ernest Kennaway.

DR. KOUWENAAR: Among the post-mortem examinations performed in Medan, 11 cases of cancer of the lung were found among Chinese against only 1 in a Javanese. These persons worked almost exclusively on tobacco plantations, but the Chinese worked part of the year in a factory where the crude tobacco was processed, and the Javanese worked all year in the fields. The Javanese women, however, work part of the year in the factory; but as a group they are much younger. It is thus possible that a correlation exists.

SIR ERNEST KENNAWAY: Is there much cancer of the lung in Bombay?

DR. V. R. KHANOLKAR: It is difficult to tell for certain. It does occur rarely, and all three types exist, but whether my figures are representative, I cannot tell.

DR. ENGELBRETH-HOLM: The latent period of human cancer being unknown, and supposed to be of considerable length, will tend to make any studies on carcinogenicity in this form of cancer difficult.

References

CLEMMESEN, J., and BUSK, T.: On the apparent increase in the incidence of lung cancer in Denmark. Brit. J. Cancer 1: 253–259, 1947.

COBBETT, L.: The Causes of Tuberculosis. Cambridge University Press, London, 1917.

DAFF, M. E., and KENNAWAY, E. L.: The arsenic content of tobacco and of tobacco smoke. Brit. J. Cancer 4: 173–182, 1950.

DOLL, R., and HILL, A. B.: Smoking and carcinoma of the lung. Brit. M. J. 2: 739, 1950.

HILL, A. B., and FANING, E. L.: Brit. J. Indust. Med. 5: 1, 1948.

Cancer in India in Relation to Race, Nutrition, and Customs. V. R. KHANOLKAR, *Tata Memorial Hospital, Bombay, India.*

Abstract. The mean annual mortality of cancer per 100,000 persons living at each age group for Bombay approximates closely the corresponding information from London and New York City.

While it is thus suggested that the susceptibility of Indians to cancer is the same as that of many other people, the incidence of cancer affecting the diverse tissues is recognizably different.

One difference ascribable to *genetical* factors is found between Habana, Cuba, and Bombay, which cities are on the same latitude, and on corresponding longitudes east and west of Greenwich. In Habana, skin cancer accounts for almost 26 percent of all cancer cases and 97 percent in white people, whereas in Bombay it accounts for only 3 percent of all cancer cases.

Furthermore, the author reported on the occurrence of penile cancer in Moslems and Hindus, and on kangri cancer. He referred to the dhoti cancer, which is associated with the wearing of a specific garment, and the chutta cancer, associated with the smoking of a homemade cigar with the lighted end in the mouth. A similar custom has been reported from Venezuela, by Wenger.

The khaini cancer is a cancer of the lower lip, showing a remarkably high incidence in Uttar Pradesh and Bihar, and is attributed to the depositing of a mixture of tobacco and lime in the lower gingivo-labial groove of the vestibule of the mouth.

Among differences in cancer morbidity due to factors the nature of which is not yet ascertained, Dr. Khanolkar mentioned that oral cancer is more frequent in the hospitals in Bombay than in other countries, and shows variations as to site, with regard to social differences. This is presumably due to the chewing of tobacco and betel with lime in certain groups of the population.

One group of population shows a remarkable frequency of cancer in the base of the tongue and in the tonsil, and a marked difference as to sex; but no explanation has yet been given this phenomenon.

Many patients with oral cancer show lower vitamin B excretion levels, even after test doses of some of the vitamin B components. Also, a peculiar dark pigmentation of the buccal and pharyngeal mucosa has been observed in several oral cancer patients.

It appears that cancer of the breast is far more common in women belonging to communities with late marriages in women and a relatively better economic position.

It seems as if primary cancer of the liver varies considerably in the different states of the Republic of India, and the number of persons suffering from this type of cancer is proportionately larger than in England or Australia. However, it is certainly not as common in India as among the Bantus in South Africa, the Negroes in French North Africa, or the Javanese.

The findings reported seem to call for a planned and intensive study of cancer in India, while conditions still permit such a study.

Discussion

MME. MUSSINI-MONTPELLIER: Findings in Africa are very similar to those in India. Both oral cancer and leukoplakia are very frequent in North Africa. Leukoplakia of the lip in Moslems is about twice as frequent as among Europeans. Leukoplakia of the tongue in Moslems is only about one third of the European rate, but the great difference is that cancer of the tongue occurs very rarely in Moslems. We find many cancers of the skin on the feet and legs.

DR. GILLMAN: Is it possible to compare statistics at all, when you have no knowledge of the normal-population statistics?

DR. KHANOLKAR: Normal-population statistics in India are available in the census reports and are fairly reliable for big cities. The difficulty lies in a correct diagnosis of visceral cancer in mortality statistics in big countries. The same difficulty exists in many other parts of the world. We have tried to compare our figures with American and Danish hospitals. In those hospitals you will find that both relatively and absolutely cancers of the gastrointestinal tract are far more frequent than in our material.

DR. DENOIX: I have found that the interest of a special hospital can largely influence the composition of the material of cases. A hospital specialized in cancer therapy will show a distribution on site quite out of proportion to reality—almost without cancers in the digestive tract, which will be treated by general hospitals—and exact age distribution can only be worked out when one knows the exact delimitation of the population served, and its distribution in age.

DR. KHANOLKAR: We realize that there are fallacies, but I still believe that the differences are sufficiently pronounced to serve as a basis for futher investigations. The question of hospital statistics has been worrying us, but so far we have no others, and with a view to the future it will certainly be of value to adopt a common but workable plan for collection of such information.

I had information from two different hospitals: The King Edward Memorial Hospital and the Tata Memorial Hospital. The King Edward Memorial is a general hospital and teaches students, preparing them for University degrees. The Tata Memorial is purely a cancer hospital. The two hospitals are separated only by a hundred yards, so they will serve very similar populations.

Regarding the question about leukoplakia, I would like to say that the leukoplakia we see in India is entirely different from the leukoplakia that one usually associates with syphilis.

DR. DENOIX: At the Paris Congress 2 weeks ago, Dr. Joyeux from Indochina made a statement of the frequency of oral cancer, and the frequency with which it is connected with the chewing of betel nut. He seems to believe that such connection between betel-nut chewing and oral cancer is only found when the betel nut is taken with lime colored red by an aniline dye, and mainly provided by burnt sea shells. It might be worth while to investigate whether there are histological or other differences in such cancers in various regions. I wonder if the lime may sometimes be quicklime and not slaked lime, thus causing burns, which in their turn may cause cancer.

DR. KHANOLKAR: Regarding betel-nut chewing, I would like to make a categorical statement, since there are many misunderstandings on this point.

A typical betel-leaf preparation is made up of a fresh green leaf from the betel plant, in which you put crushed or finely cut betel nut and slaked lime—which gives it the strong taste—and a little bit of kateku or other spice, which varies from place to place. The main composition does not vary much, although you may add more expensive spices and wrap the leaf in golden or silver foil. It has been shown in several places that where this is chewed without tobacco, there is hardly any cancer of the mouth. The lime is always slaked. In the southern part of India, along the Malabar coast, the lime is made by burning sea shells. I am of the opinion that betel leaf or other constituents play a negligible role in the production of cancer as compared to the tobacco that is chewed with it.

DR. STEWART: In some parts of the United States physicians have been impressed by the development of cancer in the parts of the mouth where tobacco chewers keep their cud.

Do people in Bombay drink alcoholic beverages?

DR. KHANOLKAR: Officially, no; there is total prohibition in Bombay at present.

DR. STEWART: And yet they have a very high incidence of esophageal cancer?

DR. KHANOLKAR: Very high.

References

KHANOLKAR, V. R.: Oral cancer in Bombay, India. Cancer Research 4: 313–319, 1944.

———: The susceptibility of Indians to cancer. Indian J. M. Research 33: 299–314, 1945.

KHANOLKAR, V. R., and SURYABAI, B.: Cancer in relation to usages. Three new types in India. Arch. Path. 40: 351–361, 1945.

KHANOLKAR, V. R.: Cancer in India. Acta Un. Internat. ctr. Cancr. 6: 881–890, 1950.

WARAVDEKAR, V. S., MANGAONKAR, V. G., and KHANOLKAR, V. R.: Vitamin B excretion in oral cancer patients. Acta Un. Internat. ctr. Cancr. 6: 1017–1022, 1950.

WENGER, F.: Personal communication.

On Cancer Incidence in Indonesia. W. KOUWENAAR, *Instituut voor tropische Hygiene, Amsterdam, Netherlands.*

Abstract. Figures were given about the incidence of cancer among the Javanese (deutero-Malay) and Chinese laborers on tobacco and rubber estates in Sumatra (In-

donesia). Some observations among Bataks (proto-Malay) of the same island were added.

1. The over-all frequency of malignant tumors is about the same as in Europe, provided that the age distribution of the population examined is taken into account.

2. In Javanese men, the majority of cancers affect the liver and belong to the hepatocellular type, usually in cirrhotic livers. In Chinese men and Bataks, this type of cancer is also very common. Perhaps the diet is responsible for this predominance of hepatic cancer.

3. In Chinese men, cancer of the stomach is about as frequent as liver cancer. In Javanese men and women, cancer of the stomach is seldom seen. The same applies to ulcers of the stomach and the duodenum. In Chinese the incidence of ulcers is about the same as in other countries, but in Javanese they are very rare. Among Bataks, however, ulcers and cancer of the stomach are regularly observed.

4. In Javanese women, cancer of the uterus and mamma are the most commonly observed malignant tumors.

5. The figures suggest that cancer of the gums and cheek is more common in a population addicted to the chewing of betel (sirih) than in other groups.

6. Cancer of the skin is usually localized on the face, the legs, and the feet.

(Discussion of this paper is combined with that of the following one by Dr. Sutomo Tjokronegoro.)

Additional Data on Cancer Incidence in Indonesia. SUTOMO TJOKRONEGORO, *University of Indonesia, Djakarta, Indonesia.*

Abstract. There is a high frequency of malignant tumors of the cervical lymph nodes among Indonesians and Chinese.

The histological picture is different from lymphosarcoma or reticulum-cell sarcoma, both of which are known in Indonesia. All pathologists there recognize these tumors as an entity, and have for lack of a better name termed them reticulo-endotheliomas. Similar findings have been reported from Malaya, Indochina, and the Philippines.

Dr. Sutomo found a high frequency of papillary cystadenoma of the thyroid gland among nonedemic goiters in Java, occurring among Indonesians, Chinese, and Dutch of both sexes. He used papillary growth—an easily distinguishable feature—as a criterion.

Among 95 nonpapilliferous goiters, there were 1 sarcoma and 3 malignant adenomas. In 9 goiters with slightly developed papilliferous growth no peculiarities were found. Of 24 goiters, predominantly or entirely papilliferous, 4 were situated at the lateral side of the neck, behind the angle of the jaw, probably arising from aberrant thyroid tissue. This makes about 19 percent papillary goiters compared with Dunhill's 0.4 percent for Europe. The same high incidence was found in the Pathology Laboratory in Semarang. Most important was the finding of definite signs of malignancy in a number of these papillary goiters, *e. g.*, infiltrative growth in neighboring tissues, invasion of blood vessels, and metastases.

Discussion

DR. OBERLING: Findings in Indochina are very similar to those in Indonesia.

(Dr. Oberling made some further comments on phimosis.)

DR. KHANOLKAR: There are variations both in the efficiency of the circumcision and in the age at which it is performed. Jewish children are circumcised during the first week, but in some parts of India and North Africa, it is not unusual to perform the ritual much later, even as late as 6 years, on Moslem children, even though the Shariya—the religious code—lays it down that circumcision should be carried out on the 7th, 14th, or 21st day after birth, not counting the day of birth.

DR. KOUWENAAR: It may be of interest, too, that cancer of the penis is not at all rare in circumcised Javanese. Doctors say that not infrequently they must operate on cases of phimosis in circumcised persons, because the original operation was made with an incomplete technique, which differs in various parts of Indonesia.

DR. GILLMAN: It should perhaps be stressed that from a nutritional point of view, Chinese brought up in China, and later working in Java, have a background different from the people born in Java, even if they take the same food now. Even the food of the mother during pregnancy may be of importance, as is the case in animal experiments. Such a factor may be a part of the explanation of the differences in the frequency of gastric cancer between Chinese and Javanese. Were there any differences in the care taken for these people?

DR. KOUWENAAR: No. We had very good opportunity for a thorough examination, because everybody incapable of working was sent to the hospital for observation by the plantation companies. Ninety or 95 percent of deaths occurred in these hospitals. With regard to background, Chinese living for many generations in Java show the same differences.

DR. KHANOLKAR: Even if Javanese and Chinese had their food made from the same raw materials, there might after all be differences in cooking.

DR. KOUWENAAR: We have tried to make clear such possible differences. Some 20 years ago the theory was raised that an ulcer might develop because food was taken very hot, and we examined the matter thoroughly but found that an inadequate explanation. Chinese will eat animal fat, mostly pork, and possibly more of it than the Javanese, who eat only vegetable fat and no pork.

DR. OBERLING: Even if they are getting the same type of food, there may still be important differences; for instance, if fat is added to the food before or after preparation. The Chinese might possibly fry the fat hotter.

MME. MUSSINI-MONTPELLIER: I wish to point out that even if nutrition is deficient in North Africa, we see very few cases of stomach cancer. Afifi from Egypt also saw many ulcers, but only few cancers of the stomach.

DR. PLATT: Dr. Kouwenaar mentioned a moment ago that phimosis, even with circumcision at 10 years, would be giving effect to the development of carcinoma of the penis many years later. Indonesian Chinese with penile cancer may thus have been born in China, and if a similar latent period is possible with regard to gastric cancer we may have to look for factors very different from those we are discussing now.

DR. GILLMAN: It certainly is an established fact that the nutrition of young animals has a great influence on their later behavior, and we know from cancer research that butter yellow acts differently if the background changes. We must be careful not to attach too much significance to any single factor in this complicated interrelation.

DR. DOLL: Have you any experience with the occurrence of pernicious anemia among the two groups?

DR. KOUWENAAR: I have never seen a patient with pernicious anemia. There is no pernicious anemia in the Javanese, and among the Chinese it is at least very rare. Although it has been stated that hypertension and angina pectoris almost do not occur among Javanese, it may be of some interest that these diseases may be found among well-to-do Javanese who have abandoned the habits of their own race, and are living more the western way.

DR. SUTOMO: In Indonesia we have tried to tackle the problem of gastric cancer and gastric ulcer. Our investigations during the period 1935–38, however, were incomplete in their scope, and we would greatly appreciate any suggestions for a practical approach to the problem.

DR. KHANOLKAR: It is interesting that stomach ulcer is very common among African Moslems, while gastric cancer is rare. Similar conditions will be found in Travancore and Madras, in the southern part of India, where the food is badly balanced and strongly spiced. There you will find a large number of juxtapyloric ulcers, but not the type localized to the body of the stomach. I wonder which type is prevalent among Moslems in North Africa.

As to hypertension, this was supposed to be extremely rare in India. Now it is increasing in frequency every year, but presumably this is due to better diagnosis, although it might be due to change of habit.

Pernicious anemia is rare, and we do not see changes in the spinal cord. Hyperchromatic anemias are extremely common.

DR. GILLMAN: With regard to gastric ulcers, we have found in South Africa that both types do occur. We have not seen angina pectoris among Africans, and there is indication that this might be due to an additional branch of the left coronary artery. Hypertension, however, does occur, and in relatively young persons. It strikes down the African at an early period, and we are deeply interested in this phenomenon. We do not see pernicious anemia.

MME. MUSSINI-MONTPELLIER: In answer to Dr. Khanolkar's question, I may say that we see all types of gastric ulcers in North Africa. I would like furthermore to call attention to the undernourishment of the Moslems there, with regard to both quality and quantity of food. There is increasing undernourishment, because of lack of meat. They do not take vegetables but live essentially on kush-kush, and are thus lacking proteins and vitamins.

DR. KOUWENAAR: The importance of malnutrition in weakening the resistance to damage is illustrated by the application in Sumatra of CCl_4 (carbon tetrachloride) against anchylostoma duodenale. Although Lambert, working in the Fiji Islands, had seen no complications, we found that the less well nourished people in Sumatra would often develop liver complications.

DR. GILLMAN: In Rhodesia it has been observed that when an African comes into the hospital and develops an acute infection, he develops jaundice very quickly. The liver is definitely vulnerable in these people.

I would like to make one further point regarding the valuation of nutrition. It has, for example, been demonstrated that even if the food consumed by a person during a single day may seem to be more than adequate, it may finally prove insufficient, because of its administration. Thus, if two out of four meals contain all the fats and carbohydrates, and the other two meals the high fats and high proteins, then the individual may go into negative nitrogen balance. In 1925 Jackson found differences in the relative weight of organs, especially with regard to the pancreas. From our studies in Africa we think that malnutrition will influence the organ weights.

DR. KHANOLKAR: The relative weights of internal organs in Indians is not in conformity with those of the Europeans.

DR. GILLMAN: It may be worth while to study cancer of the pancreas more closely. From the Mulago Hospital in Kampala, Uganda, a relatively large number of pancreatic cancers have been reported. They seem to have a high incidence of pancreatic fibrosis.

References

BONNE, C.: Cancer in Java and Sumatra. Am. J. Cancer 25: 811–821, 1935.

———: Cancer and human races. Am. J. Cancer 30: 435–454, 1937.

———: Le cancer dans les pays tropicaux de l'extreme Orient. Compt. rend. de X^me Congr. de la F. E. A. T. M. Hanoi, 1938.

BONNE, C., HARTZ, P. H., KLERKS, J. V., POSTHUMA, J. H., and RADSMA, W.: Morphology of the stomach and gastric secretion in Malays and Chinese and the different incidence of gastric ulcer and cancer in these races. Am. J. Cancer 33: 265–279, 1938.

FOSSEN, A.: Over maligne halslymphkliergezwellen. Thesis. Batavia, 1936.

SUTOMO, T.: Pathologisch-anatomische bezonderheden over strumae uit de Heelkundige Kliniek te Batavia. Genees. tijdschr. v. Nederl.-Indië 81: 515–544, 1941.

Cancer in North Africa. MME. J. MUSSINI-MONTPELLIER. *Centre algerien de lutte contre le cancer, Alger, Algeria.*

Abstract. Mme. Mussini-Montpellier described the climatical and ethnological background of the studies on cancer in North Africa, carried out by J. M. Montpellier and J. Mussini-Montpellier, and presented a series of observations. These were set

forth not as established facts but as "provisional" facts, which may serve as working hypotheses. The following list represents only a part of the observations.

There is an increase in the relative rate of sarcomas and also of malignant melanoblastomas for both Europeans and Moslems in proportion to distance from northern latitude and nearness to the tropical and subtropical zones. Similarly, there is a frequency of cancer of the liver, already quite marked, in North Africa and considerable in the tropical zone and the parts of Africa that lie in the Southern Hemisphere.

There is an inversion of the characteristic distribution of skin cancer in tropical Africa by comparison with that of Europe, and this inversion begins in North Africa.

There is a decrease of the frequency of cancer of the uterine body in comparison with cancer of the uterine cervix, and there is a decrease in the frequency rate of cancer of the breast in the female, whereas among males this condition is more pronounced in North Africa than in Metropolitan France.

Cancer of the tongue, the stomach, the intestine, the lung, and the prostate seem relatively rare.

On the whole, apparently cancer develops at a younger age in the African than in the European.

In our statistics cancer of the larynx ranks third in frequency after the skin and the genital organs. It may be discussed whether this may be ascribed to syphilis or to tobacco, or to other factors.

Discussion

DR. SYMEONIDIS: I was wondering at the apparently normal frequency of skin cancer among the European population in North Africa. In Greece, skin cancer is frequent, presumably due to the intensity of the sunlight.

MME. MUSSINI-MONTPELLIER: I can only repeat that the total frequency for skin cancer among Europeans is the same as in France, the distribution being 80 percent in the face and 10 percent in other regions of the body.

DR. DENOIX: It would be interesting to compare the inhabitants of Southern France with those of North Africa, but I am afraid that that is impossible at the moment.

DR. MAISIN: Perhaps the European population living in North Africa have been forced to protect themselves more efficiently to the effects of sunshine than the Greeks.

DR. STEWART: I understand that there are two groups of Jews within the area studied. Were there any differences with regard to religious rite or economical status between these two groups?

MME. MUSSINI-MONTPELLIER: We are aware of the interesting problems connected with cancer among Jews, but we would like to have more information on the Jewish population. The only differences suspected so far are, however, regional.

DR. KHANOLKAR: I would like to demonstrate a graph on the morbidity data at the Cancer Hospital in Bombay, a seaport with a big textile factory center. Among the Deccani Hindus, who supply most of the textile labor and are economically poor, almost 50 percent of the female cancer patients were suffering from cancer of the cervix. The Parsees, an economically far better situated group, showed, however, almost 50 percent of breast cancer among the female cancer patients. Twenty years ago it was demonstrated in a paper by Nath and Greval that India showed a far higher ratio of sarcomas to carcinomas than Europe. Our data do not support this statement. A diagnosis of sarcoma on any rapidly growing tumor on clinical and sometimes even histological examination is a common error. It needs a little experience to differentiate a very anaplastic carcinoma from a sarcoma.

On the whole the results presented by Dr. Mussini-Montpellier leave us in no doubt as to the necessity of further studies, and the excellent conditions afforded by the mixed populations in North Africa.

DR. CLEMMESEN: It would be highly interesting if, for instance, Jews in Morocco show a higher apparent frequency of cervical uterine cancer than Jews in Tunisia. I believe that it can be considered as practically demonstrated that Jewish women,

whatever the reason, show fewer cases of cervical cancer than other women, except nuns who do not suffer from this disease (Gagnon 1950). In 1939 G. Wolff showed that where allowances for differences in average age distribution is made, the Jewish population in Berlin showed fewer deaths of uterine cancer than other Germans. At the conference on cancer statistics in Copenhagen, in 1946, material from Palestine was presented, collected for the Danish Cancer Registry by Dr. Holger E. Holm: The Beilinson Hospital in Petah Tikwa in the period from 1936 to 1946, while serving a population of 150,000 to 250,000, treated 1 case of cervical cancer and 25 cases of cancer of the uterine fundus. In the same period the number of breast cancers amounted to 146; the Municipal Hadassah Hospital in Tel Aviv, serving a population of about 200,000 from January 1943 to May 1946, treated 1 case of carcinoma simplex of the cervix, 5 cases of carcinoma of the body of the uterus, and 37 cases of breast cancer. These figures suggest that it is the cervical cancer that fails to develop in Jews, because the proportion is almost the reverse of what is usual in Europe. However, it must be remembered that because of the earlier average occurrence of cervical cancer in relation to fundus cancer, an aging population as in Denmark or Sweden will show a relatively increasing amount of cancers in the uterine fundus. So, without information on the age distribution of the population, we cannot accept evidence as final. However, I find it utterly unlikely that the Jewish population should have an age distribution that could explain the findings mentioned. We had started preparations for a comparative study with Dr. Doljanski and communicated with Dr. Bacchi, but then the war came. Considering the causes of this "racial" difference, it is interesting that while fundus cancer shows a tendency to familial occurrence, cervical cancer seems not to be inherited (Bröbeck 1949—Clemmesen 1949).

DR. SYMEONIDIS: Did you have any information on the general population in Tel Aviv?

DR. CLEMMESEN: No. Dr. Karplus, who was at that time pathologist to the hospital, told us that reliable information on age in Palestine was extremely difficult to obtain. The immigrants would very often be ignorant of their real age. Furthermore, they want to make an entirely new life, and often change their names for this reason, which does not make it easier to check their age. I think it should also be noted that there is more than one race of Jews.

DR. MAISIN: Dr. Hochmann has produced statistics from the Cancer Institute in Jerusalem. He stated that for the period 1933 to 1947 they saw 81 cervical cancers for every 100 of the fundus among Jewesses, while Armenians showed 105 cervical cancers to 9 of the fundus.

DR. SYMEONIDIS: From the National Cancer Institute in Bethesda we have established contact with officials in Israel, in order to establish a thorough investigation on the subject. We, too, realize the shortcomings of hospital statistics, and would welcome a well-founded statistical inquiry. We have every hope of collaboration with Israel authorities.

DR. KHANOLKAR: As a fact of possible interest in this connection, I would mention that since about 800 A. D. a Jewish population has lived isolated on the Western coast of India. They are called the Ben-Israels, and they would now number about 10,000.

DR. CLEMMESEN: I would recommend strongly that any future investigations on uterine cancer or on breast cancer should deal also with the other type of cancer. There is ample evidence that when nativity increases we see an increase of cervical cancer, and a corresponding decrease in breast cancer. Also, studies on the social distribution of these cancers seem to demonstrate an antagonistic interrelation between them. It would be extremely interesting to see whether this is due to a single factor, for instance, a hormone, or to two independent factors that frequently coincide. It would certainly be important to know if breast cancer is commoner than would be expected among Jewesses—and just as interesting to know for certain if it shows the same incidence as in other populations. The amount of work involved in examining the frequency of two cancers is not twice the work entailed in working on a single type,

because the main work is the establishment of figures for the normal population. And when it is the question of a comparison, the work has to be carried out simultaneously for both cancers.

In this connection I would like to ask Dr. Mussini-Montpellier if anything is known as to nativity among the various races in North Africa. For some of the races it seems as if breast cancer and cervical uterine cancer show just the relationship you would expect from a difference in nativity.

DR. SUTOMO: In connection with the observations of Dr. Mussini-Montpellier on carcinoma of the corpus uteri in North Africa, I should like to give some figures from my paper on the incidence of uterine cancer among the different racial groups in Indonesia (Sutomo 1937). In a histological study of biopsies and surgical specimens received in the Department of Pathology of the School of Medicine in Djakarta (Batavia) during the years 1927 to 1937, of which the majority were sent by the Department of Obstetrics and Gynecology of the Central Civil Hospital in the same city, I found three striking facts:

1. The low percentage (2.9) of cancers of the uterine body. This low percentage contrasts with the figures given in the literature of Western countries (6-17 percent). From the clinical side, similar low figures have been published by Remmelts in 1935.

2. The relatively high percentage of cervical cancers with a papilliferous structure (18 out of 20) and of typical cervical cancers in general (6.9 percent), also contrast with average figures given by Pankow; Laborde and Wickham; Laborde, Leroux and Millot; Leroux and Millot; and Healy.

3. The frequency of uterine cancer as a whole among the Chinese. This is even more striking if we take into consideration that there are approximately 1.6 million Chinese against 70 million Indonesians, and that the average number of Chinese in the above mentioned hospital is one-third or less the number of Indonesians.

DR. KOUWENAAR: In answer to Dr. Maisin's question regarding the different frequency of genital cancers in Chinese and Indonesian women, I would like to refer to statistics by Remmelts from the Gynecological Department in Djakarta (Batavia) from which it appears that genital cancer is more frequent in Chinese than in Indonesian women. However, the age distribution is not given.

SIR ERNEST KENNAWAY: Are the Chinese more urban dwellers than the Malay?

DR. KOUWENAAR: Yes, at least in Java. On Sumatra's eastern coast many are working in the fields.

SIR ERNEST KENNAWAY: Is there any reluctance on the part of the Indonesians to subject themselves to examination for genital diseases?

DR. SUTOMO: Usually not.

DR. KOUWENAAR: I would like to ask Dr. Mussini-Montpellier if yaws is seen in North Africa.

MME. MUSSINI-MONTPELLIER: No, neither in Moslems nor in Europeans.

DR. KOUWENAAR: In Indonesia we have noticed a connection between the site of the scar left by yaws and the location of the skin cancer. In regard to cancer of the skull, I have found this cancer frequently in Javanese and Chinese.

SIR ERNEST KENNAWAY: Is cancer of the larynx equally common among males and females in North Africa?

MME. MUSSINI-MONTPELLIER: In Algiers we have not seen this cancer in women, but we see a large number of cases in men.

DR. MAISIN: But would women seek hospital for this cancer?

MME. MUSSINI-MONTPELLIER: Oh, yes.

SIR ERNEST KENNAWAY: Do women smoke in North Africa?

MME. MUSSINI-MONTPELLIER: No; never.

DR. J. GILLMAN: I would like to call attention to the question of the age of patients. In South Africa we have the impression that the usual morphological and physiological criteria for age in the African are different from the European. We see a delayed puberty and an early menopause. It is rare for a woman over 30 years of age to have more children. Also diseases like hepatic fibrosis seem to occur at an earlier age than

among Europeans. We have seen a case of liver carcinoma in a child of 14, and over a short period no less than 5 of 20 cases of carcinoma of the liver occurred in individuals under 20 years of age.

This altered reactivity we regard as a manifestation of a speeding of the life-track of malnourished Africans. This speeding up does not imply increased thyroid activity but rather a complete change in the metabolic processes. Such differences in reactivity are deep-seated and originate most probably *in utero*, consequent to the altered metabolism of the mother, induced by chronic malnutrition.

My brother and I have examined this aspect in considerable detail, and the results are incorporated in a book now in press.

It may be of interest to mention that before disintegration of tribal life, the African was always regarded as virile and in an excellent state of health. However, the rapid industrialization associated with unplanned urbanization and shanty towns have led to great poverty. Twenty-five years ago, pellagra was rare, and now we ourselves, with limited facilities, have been able to collect records of 2,000 cases within a single year.

DR. PLATT: I would like to add some evidence in order to avoid the impression that the early menopause of the African is due to any increased intensity of metabolism. Just recently we have completed a series of metabolic balances on apparently normal African children, normal by local standards. They were allowed to eat the food they liked and the quantities they wanted. The nitrogenous metabolism in children 6, 10, and 12 years of age is less than in American children at the age of 4 years. Thus, there is a general lowering of metabolic activity, which may well have pathological effects.

DR. CLEMMESEN: On liver cancer I should quote G. M. Findlay: Medical examination of 227,000 soldiers mainly from rural W. Africa aged between 18 and 40 showed 36 primary hepatomas, 1 bile duct carcinoma, and 23 other cancers. Twenty-three cancers amount to 10.1 per 100,000 soldiers, while 50,000 European troops in the same area showed 12 per 100,000 and no liver cancers. It would seem at a glance that the total rate for the Negro, in spite of the large number of hepatomas, corresponds better with European rates than that of the white soldiers.

DR. SYMEONIDIS: Clinical and autopsy material from Greece suggests an increased frequency of liver cancer. In 3,828 autopsies, we found 381 cancer cases of which 41 were primary cancers of the liver. The percentage of male liver cancers was 1.4 and of female 0.3, and 61 percent were associated with cirrhotic changes. The age-distribution curve seems to have its peak between 40 and 60 years. Even if we allow for these figures being collected from autopsy material, I think they suggest that Greece, where malnutrition is to some extent endemic, shows a higher frequency of primary liver cancer than other European countries. Corresponding figures from Kampala, Uganda, were 2,791 autopsies, with 1.2 male and 0.5 percent female cases of primary liver carcinoma. Further comparisons would seem interesting (Papelia 1940).

DR. GILLMAN: Also, the incidence of gynecomastia suggests abnormal hormonal changes probably due to malnutrition. We know from the American prisoners starving in Japanese prison camps that the incidence of gynecomastia increased. The question arises whether the high incidence of gynecomastia in Africa may not be of significance in the etiology of carcinoma of the liver.

DR. SYMEONIDIS: In Greece, where the poststarvation gynecomastia was first described we saw the same conditions as in the Japanese prison camps, namely, a long period of starvation followed by sudden intake of more or less adequate quantities of food. In consideration of the possible relationship between this condition and breast cancer in Africa I shall later give a report on the subject.

I would like to add that during the period mentioned menostasis was extremely common. It was my experience that fibroadenomatosis of the breast was very frequent too, and perhaps a study of the occurrence of breast cancer in the years to come might be of interest.

DR. DENOIX: In a systematic study of 20,000 inhabitants of Haute Volt in French West Africa I saw practically only keloid reactions in persons with an increased volume of the thyroid gland of varying degree. These indications of a hypofunction of the gland were very frequent in the group examined. (For 100 cases of keloid, females showed 91 and males 63 cases of abnormal thyroid.)

MME. MUSSINI-MONTPELLIER: In North Africa I have seen a rather pronounced tendency to the development of keloid in scars, and I have also seen many goiters.

DR. KHANOLKAR: India has seen a rapid urbanization taking place. Before World War II, Bombay had 1.5 million inhabitants, but the population has now increased to 3.5 million inhabitants within the last few years. The population of Calcutta has nearly tripled since the separation of Pakistan. Unfortunately, this raises a question of malnutrition and density of population, because it is difficult for these people to find jobs. It would seem that investigations carried out on the lines indicated by the results of our colleagues from Africa might give interesting results. But it would certainly be advisable that such investigations should start soon in order to take advantage of the present conditions.

DR. ENGELBRETH-HOLM: I would like to be informed whether it is still believed that the reason for liver damage, with consequent increase of certain cancers and gynecomastia after malnutrition, is that the damaged liver cannot metabolize the estrogen. The result of this could be an increased estrogen level, which could explain the cancers and the gynecomastia observed, whereas the liver cancers would be a different consequence of the malnutrition.

DR. GILLMAN: The relationship between estrogen metabolism and liver pathology is by no means as clear as some investigators would believe. We have attempted a correlation between the extent of liver injury and enlargement of the breasts in the male, or disturbance of the menstrual cycle in malnourished African women. We could not establish a positive correlation. Moreover, we do not believe that the inference drawn from the effect of pellets of estrogen implanted in the spleen of animals on deficient diets or poisoned with carbon tetrachloride can be compared with the effects of malnutrition alone. It is known that in chronic malnutrition there is a general depression of pituitary activity, so that gonadal depression is to be anticipated. However, in the males, the androgens seem to be depressed first and the estrogen secretion persists but is not in any way intensified. This does not imply that the estrogens are abundant as is evident from our investigations in male baboons. Moreover, we have demonstrated that the androgen dominance in male baboons can be readily suppressed, and in some of our male baboons we have induced gynecomastia within 5 to 7 days by intense irritation of the reticulo-endothelial system.

DR. LACASSAGNE: I realize the great interest of the statistics which Dr. Mussini-Montpellier has presented in such a brilliant way; however, I would like to indicate that I find it may be dangerous to draw conclusions from them—perhaps not with regard to the differences between European and Moslem population in Africa, but certainly with regard to the differences between the populations of France proper and North Africa.

In fact, it is necessary not to forget the fundamental part played by the age of the patient, both with regard to cancer in general and with regard to cancers of every single site. We do know that the age-distribution curves of the various types do not coincide, and that there are some cancers that are more prevalent among the younger age classes, while other types show a more advanced average age.

The numbers given for the frequency of cancers of various sites have only their comparative value for populations for which the age distribution is known for decennial classes of age. Since such knowledge is not available for North Africa, a comparison of the incidence of that area with France proper, where the average age is far higher, runs the risk of showing erroneously a higher percentual occurrence of those cancers that are more likely to develop in younger individuals, and a reduced frequency of those cancers that most often develop in older persons.

MME. MUSSINI-MONTPELLIER: The European population in North Africa will show the same age distribution as in France. I would point out that we have no military personnel included in our statistics, but, of course, the data for age are not entirely reliable, so that an elaborate comparison of age-distribution curves will be impossible at the moment. Nevertheless, if you have 25 cases aged below 20, and 23 of them are Moslem and only 2 are European, in a place where there are more Europeans than Moslems, I believe that the difference may be considered as striking.

In this connection I would like to say that also in North Africa, as in India and South Africa, aging comes earlier than in Europe.

DR. CLEMMESEN: With regard to the importance of statistical evidence, I think that we should not fail in making requirements strict, when we want to regard a case as finally proved. In Denmark we have spent much time on working out correct and detailed age-distribution figures, in the hope that we may be able some day to work out comparisons with other countries, preferably as remote from Europe as possible. Such comparisons are naturally more likely to be efficient as far as urban areas are concerned. It has to be realized, however, that it is most difficult to obtain reliable curves from extensive countries, where hospital statistics are the only reliable sources of information on incidence and most often not backed up with detailed population statistics. The applicability of such age-distribution curves will largely depend on the extent to which the age distribution of tumors will vary with etiology. It has, for instance, been demonstrated that lung cancers in Joachimsthal—caused by the work in the uranium mines—appeared earlier in life than lung cancers in New York. It would definitely be important if similar differences could be demonstrated between, for instance, liver cancers occurring in Africa and in Europe.

But even if the statistical study based on accurate figures will have the final say as to the reliability of results, it would certainly be dangerous to rule out results because they may be subject to statistical doubts. I personally am very grateful to Percivall Pott that he did not wait for perfect age-distribution figures before he stated that scrotal cancer was more frequent among chimney sweeps. I think also that imperfect statistics may have their value as an inspiration, and would like to point to the way in which some experimental results are taken into consideration even if they are not regarded as being the final proof. If only experimental workers would take the statements from what is called the statistical side in a similar way, and try to make a more graded evaluation, it would be useful. I would like to join Dr. Lacassagne in his skepticism about percentual figures when they have not been adjusted for differences in age distribution, but I would not deny that such statements as Dr. Mussini-Montpellier's, may be of great value as an inspiration for further work.

DR. MAISIN: I would like to ask Dr. Dorn if he has found any difference in the occurrence of skin cancer in the Northern and Southern states, in America, with regard to age distribution.

DR. DORN: No. We have seen a difference in incidence, but not in age distribution. In countries like India or Africa, with a population that is generally younger, one would naturally expect that the cancer cases occurring will also be of a younger age, because the older persons who contribute the larger part of European cancers simply do not exist. Thus, hospital statistics will give a picture of young cancer cases, if they do not pay attention to the age of the population served. If it were not so, it would be surprising. It may well be that biological aging proceeds more rapidly in those areas, and there might well be a difference in age distribution, but all I am saying is that the statistical data here presented so far are insufficient to prove that.

References

BRÖBECK, O.: Heredity in Cancer Uteri. (Danish Thesis) Aarhus, Universitetsforlaget, 1944. P. 100.

CLEMMESEN, J.: The status of genetical studies in human cancer. Brit. J. Cancer 3:474–484, 1949.

FINDLAY, G. M.: Observations on primary liver carcinoma in West African soldiers. J. Roy. Micro. Soc. 70: 166–172, 1950.

GAGNON, F.: Contribution to the study of etiology and prevention of cancer of the cervix of the uterus. Am. J. Obs. & Gyn. 60: 516–522, 1950.

MONTPELLIER, J. M., and Mussini-Montpellier, J.: Cancer en France d'outre mer. Paris, 1947.

———: Cancer en Afrique du Nord Francaise. Maloine. Paris, 1950.

PALES, L.: Les tumeurs des indigènes en Afrique Noire. Méd. Trop. 1: 289, 1941.

———: Le probleme des Chéloides et le point de vue tropical. 2: 183 and 275, 1941.

———: Cancer et races. Bull. Medic. AOF, Dakar. 3: 73, 1946.

———: Le Cancer au Soudan (Rapport). Bull. Medic. AOF, Dakar 3: 109, 1946.

PAPELIA, D.: Cancer of the liver (Greek). Athens, 1940, Diss.

REMMELTS, R.: One hundred cases of uterine cancer in Batavia. Geneesk. tijdschr. v. Nederl.-Indië 75: 1287–1301, 1935.

SUTOMO, T.: Adenocarcinoma of uterine cervix. Geneesk. tijdschr. v. Nederl.-Indië 77: 3140–3149, 1937.

WOLFF, G.: Cancer and race, with special reference to the Jews. Am. J. Hyg. 29: 121–137, 1939.

Conditions for the Registration of Cancer Cases in French W. Africa. P. F. DENOIX, *Institute National d'Hygiene, Paris, France.*

Abstract. After having pointed to the considerable interest of comparisons of cancer among Negroes in different parts of the world, the author commented upon the special difficulties involved in the registration of cancer cases in French Africa.

These difficulties are due to: lack of demographic information; lack of any information on age of Negroes; scarcity of the medical profession; and, pronounced variety in the climate of the extensive regions involved.

These difficulties are now under consideration with the object of diminishing their influence. The provisional statements made are based on notifications from all the French Medical Corps in French West Africa, who report all cases of cancer they encounter. The number of cases increases every year. Some provisional figures may be given on the special frequency of cancer in certain sites for men and women, and they confirm the very special character of the distribution of cancer by site in the population concerned. In concluding, the author gave some indications as guidance to those who want to start investigations in similar regions.

Discussion

DR. PLATT: Will it be possible on the basis of Dr. Denoix's experience to develop a technique to obtain reliable data from primitive populations also; and, for instance, to distinguish between urban and rural areas?

DR. DENOIX: It would take several years to obtain accurate information on the demography of French West Africa, but in the meantime one can develop the registration of cancer cases through all the members of the medical profession in a given area. We have allowed for the unknown number of cases of deeply sited cancers that will escape such an investigation. It may be possible to obtain interesting information in this way.

DR. CLEMMESEN: May it not be possible to develop a rough age grouping based on outstanding physiological data, such as puberty, childbearing, and menopause?

DR. DENOIX: We have, of course, tried to do it, but the only thing we could do was to exclude persons under 25 years of age, and this is not of much value.

DR. PLATT: Is it then worth while to collect the facts, if age grouping is impossible?

DR. DENOIX: It is impossible to obtain exact precision, but one may collect valuable information.

DR. VISSCHER: I would recommend that the conference divide the survey data into: definitive data, and indicative data.

Each type should have a special usage: one should indicate where more critical studies might be made, and support should be obtained if studies are not too expensive.

DR. GILLMAN: All information on the statistical distribution of cancer can be very valuable, as has been shown in the relatively high incidence of cancer of the liver disclosed by investigators working in different parts of Africa, and under great local difficulties. The experts, like Drs. Clemmesen and Dorn, are, I think, sufficiently acquainted with the various problems of documenting statistics in different parts of the world, whether primitive or sophisticated, and they could give practical guidance for future statistical investigations for use in primitive communities.

DR. PLATT: I want to add that I recall that the FAO has met with similar difficulties in obtaining data, in order to make out comparable areas. I am sure that these agencies have worked out a system that could be adopted.

DR. STEWART: Are those cases cited by Dr. Denoix derived from hospitals or from reports by physicians, or from both?

DR. DENOIX: They come not only from pathological laboratories but also from the medical profession in the areas concerned.

DR. ENGELBRETH-HOLM: That is important. Judging from autopsies alone, or from biopsies, would, at least in the hospitals of Europe, give an entirely wrong impression of the situation. From a radium hospital the pathological material will show a high percentage of uterine cancers and skin cancers, but next to no cases of the stomach or the intestine. On the other hand, a pathological institute in a municipal hospital may give a high percentage of gastric cancers but no skin cancers or uterine cancers.

DR. OBERLING: How was the diagnosis made of liver cancer—by puncture?

DR. DENOIX: The clinical diagnosis of primary liver cancer does not present itself in the same way in French West Africa as in Europe. In Africa, it is almost a nearly common clinical syndrome, easily recognized. On the other hand, the development is very rapid, death in the advanced cases following 2 or 3 months or a few weeks after hospitalization. The larger part of our cases have been checked by autopsy and histological examination.

DR. GILLMAN: Liver cancers are also very common in the hospitals in South Africa, and easy to diagnose.

DR. KHANOLKAR: I would like to add that even if it is desirable that doctors should report on the cases, not all doctors are of the same quality, and it may well be that a scheme that would work well in Europe or the United States, would fail to give accurate information from Indonesia or Africa. I, too, think it desirable to work out schemes for different levels of medical development.

(The discussion ended in remarks on the various possible procedures. It appeared that Drs. Denoix, Stewart, and Symeonidis had planned to create a team consisting of one pathologist, one expert on nutrition, and one statistician, for studies in Africa.)

Observations on the Etiology of Cancer of the Liver. (J. GILLMAN, T. GILLMAN, and C. GILBERT) JOSEPH GILLMAN, *Department of Physiology, University of the Witwatersrand, Johannesburg, South Africa.*

Abstract. The authors reported on the distribution of cancer in various parts of Africa and found it improbable that it was conditioned by either genetic or climatological factors, while dietary factors in carcinogenesis were discussed more in detail for the following reasons: 1) A high incidence of liver and pancreatic damage can be produced experimentally by nutritional technique. 2) The rate of development of carcinoma induced experimentally by carcinogens may be retarded or expedited by varying the diet. 3) It has now been shown, especially in South Africa, that malnutrition is associated with a high incidence of hepatic damage and in Uganda with disease of the pancreas and of the liver. 4) The high incidence of hepatic damage in the South African native tends to be associated with carcinoma of the liver. In the native of Uganda, pancreatic disease as well as liver disease is frequent, and there is a relatively high incidence of liver and pancreatic cancer.

Other details on the distribution of cancer in Africa were that hepatic carcinoma occurs three times more frequently in the Mozambique natives than among natives in the Union of South Africa. In the urban African, cancer of the stomach is seen more often than in the male African on the gold mines.

Other points of significance are:

Among hepatomas artificially produced in rats, the authors saw no case where some degree of bile-duct proliferation was absent. Close examination of the material disclosed that the distinction between hepatoma and cholangioma is arbitrary, since all transitions can be found between liver and bile-duct cells. Also, in some human cases bile-duct proliferation can be very extensive, and in many cases the authors found all gradations between cells resembling those found in Hering's segment of the bile duct and liver cells.

Animal experiments showed that ligation of the common bile duct is attended by a variable degree of necrosis and by extensive replacement of hepatic parenchyma by proliferating duct epithelium. The authors have seen similar pathological changes in complete obstruction of the bile duct in man. On special diets rich in carbohydrates, moderately rich in proteins but extremely low in fats and devoid of fat-soluble vitamins, rats develop a low-grade necrosis often attended by extensive proliferation of bile ducts instead of fibroblasts, and sometimes by the presence of small calculi in the bile ducts. In some experiments bile-duct hyperplasia was so extensive as to suggest a precancerous change. The authors have also demonstrated that necrosis of the liver will be complicated by fibrosis more frequently if the animals are fed special diets.

Further experiments showed that disturbances of bile and pigment metabolism induced by dietary technique would often lead to rapid atrophy of the testis and consequently influence the metabolism of steroid hormones.

There are indications that bile metabolism is modified in the African. For instance, there is an excess of porphyrins in the liver in malnourished individuals, and the metabolism of these compounds can be influenced by short exposure of the skin to ultraviolet light. The metabolism of vitamin A, supposed to be a chologogue, and of bile, can be influenced by the reticulo-endothelial system, which, in turn, may be irritated by malarial and other parasitic infections. Irritation of this system tends to be associated with adrenal hyperactivity and depression of gonadal function. The frequency of testicular atrophy and gynecomastia, with or without gross liver disease, in malnourished African males may also find a place in the complex chain of events culminating in carcinoma of the liver. Malnutrition is also associated with depression of thyroid activity, a fact which may not be without interest in view of the production of hepatic carcinoma by thiouracil.

The authors suspected as far as the African native is concerned that the high incidence of liver disease, disturbance in the metabolism at least of bile, vitamin A, and the sterol hormones, and to a lesser extent of the thyroid, are most deeply implicated in the production of carcinoma of the liver.

Discussion

SIR ERNEST KENNAWAY: Do these Negroes live in reserves or in the towns?

DR. GILLMAN: It is chiefly the question of urban population around Johannesburg, but the single individual will often try to keep his origin secret, because of the restrictions on settling.

DR. PLATT: In my mind it is not unlikely that there is some connection between malnutrition and cancer. But I would like to make a few points that may be of importance to those mainly acquainted with studies on nutrition in Europe.

I would like to point out that the balance of the diet is not just one of protein to carbohydrate, because the demands on the protein in the body will vary with various exogenous factors, and this is important in Africa where there are no stable dietary conditions as in the sophisticated communities. Some studies of weight curves from African children illustrated this clearly through constant oscillations of the ascending curves. At a certain time of the year, just when food is running short, people have

to get the next year's harvest in, so that requirements go up while intake is going down, and at the same time parasitic infections are occurring, which affect the proteins available in the body.

DR. GILLMAN: I would also like to stress that animals fed on the same food may utilize it in different ways, so to speak, with a different metabolic machinery. This might even apply to people of various somatic types.

DR. MAISIN: Also restricted caloric intake will, if judged from experimental evidence, play some part with regard to carcinogenesis.

DR. SUTOMO: In Indonesia we have always been struck by the fact that patients suffering from such a severe and serious disease as primary cancer of the liver are often in very good condition even a few days or a few hours before death.

I should like to ask Dr. Gillman whether his experience is similar with cases of primary liver cancer in South Africa, and, if so, what is his explanation of this striking contradiction?

DR. GILLMAN: Dr. Berman, who has carefully studied the clinical course of hepatic carcinoma, has stated that some cases of hepatic carcinoma may remain in very good condition until a short time before death.

DR. DENOIX: Nutritional conditions in French West Africa seem very different from those described by Dr. Gillman for South Africa. In French West Africa the population is essentially rural, and there is no industrialization nor any urbanization of any importance. The level of nutrition is slightly inferior to the normal, but not qualitatively irregular. Pellagra or signs of other avitaminoses have not been observed. The only period in the life of the Negro where there is an important fasting period is immediately after cessation of nursing when the child is given the same food as the adult, which it cannot assimilate properly. The child cannot walk yet, and thus is unable to add the vitamins of fruits or berries mellowing on bushes. This period will on the average extend between the ages of 3 and 5 years.

The conditions are thus different from South Africa, but liver cancer is also frequent in French West Africa.

DR. VISSCHER: Dr. Gillman's presentation emphasizes the stages of refinement of work that grow out of endemiological studies in cancer. The high incidence of liver cancer in Africa pointed to the desirability of study of related circumstances, which in turn led to suggestions for basic biological research.

I hope that similar analytical studies may evolve out of observations on the circumstances surrounding high incidences of other types of cancer in other cases.

References

BERMAN, C.: Malignant disease in the Bantu of Johannesburg and the Witwatersrand gold mine. South African J. M. Sc. 1: 12–30, 1935.

———: Primary carcinoma of the liver in the Bantu races of South Africa. South African J. M. Sc. 5: 54–72, 1940.

———: The clinical features of primary carcinoma of the liver in the Bantu races of South Africa. South African J. M. Sc. 5: 92–109, 1940.

———: Etiology of primary carcinoma of the liver with special reference to the Bantu races of South Africa. South African J. M. Sc. 6: 145–156, 1941.

BURROWS, H.: Biological Action of Sex Hormones. Cambridge University Press, 1945.

DAVIES, J. N. P.: Pathology of Central African natives; Mulago Hospital post mortem studies, 6. Cancer in Africans. East African M. J. 25: 228–235; 322–326, 1948.

FOGELMAN, M. J., and IVY, A. C.: The effect of thiouracil on liver regeneration. Am. J. Physiol. 153: 397–401, 1948.

GILLMAN, J., GILLMAN, T., and BRENNER, S.: Vitamin A and porphyrin-like fluorescence in the livers of pellagrins, with special reference to the effects of a high carbohydrate diet and methionine. South African J. M. Sc. 10: 67–80, 1945.

———: Porphyrin fluorescence in the livers of pellagrins in relation to ultra-violet light. Nature 156: 689, 1945.

GILLMAN, J., GILLMAN, T., and GILBERT, C.: Reticulosis and reticulum-cell tumors of the liver produced in rats by trypan blue with reference to hepatic necrosis and fibrosis. South African J. M. Sc. 15: 21, 1949.

———: Lymphomata (including Hodgkin's-like sarcomata); their experimental production. Clin. Proc. 8: 4, 1949.

GILLMAN, J., and GILLMAN, T.: Perspectives in Human Malnutrition: A Contribution to the Biology of Disease from a Clinical and Pathological Study of Chronic Malnutrition and Pellagra in the African. Grune and Stratton, New York, 1950.

LOPIS, S.: The incidence of cholelithiasis in the Bantu. Clin. Proc. 6: 338-347, 1947.

MASON, K. E.: Differences in testis injury and repair after vitamin A deficiency, vitamin E deficiency and inanition. Am. J. Anat. 52: 153-239, 1933.

OPIE, E. L.: The pathogenesis of tumors of the liver produced by butter yellow. J. Exper. Med. 80: 231-246, 1944.

ORR, J. W.: Histology of rat's liver during course of carcinogenesis by butter yellow (*p*-dimethylaminoazobenzene). J. Path. & Bact. 50: 393-408, 1940.

PRATES, M. D.: Primary malignant tumors of liver in Mozambique natives. South African M. J. 14: 95-99, 1940.

SIMSON, F. W., and STRACHAN, A. S.: A study of experimental tissue reaction following intravenous injections of silica and other dusts. Publications of the South African Institute for Medical Research No. 45, 9: 95, 1940.

SOBOTKA, H.: Physiological Chemistry of the Bile. Bailliere, Tindall & Cox, London, 1937.

Poststarvation Gynecomastia and Its Relationship to Breast Cancer in Man. A. SYMEONIDIS, *Geographical Pathology Unit, National Cancer Institute, Bethesda, Maryland.*

Abstract. Based on material from Athens (1943 to 1944) obtained by biopsy, Dr. Symeonidis has collected 107 cases of gynecomastia from all parts of Greece. Gynecomastia was found unilaterally in approximately 75 percent of the cases.

The frequency seems to be most pronounced in the periods of life when sexual function is labile.

A remarkable increase seems to have occurred in the years 1943 and 1944, at a period when starvation itself was on the retreat.

Dr. Symeonidis demonstrated photographs and photomicrographs. He ascribed the development of this condition to the effects of famine, during which hormonal activity is reduced, and the following sudden function of the endocrine organs immediately after adequate intake of food. The hormonal aspect of this mechanism was also discussed with a view to the fact that breast cancer in males is particularly frequent in Africa, where chronic malnutrition is endemic in many places. It is to be hoped that malnutrition at one period will not have the same effect as chronic malnutrition.

References

SYMEONIDIS, A.: Unusual appearance of many cases of gynecomastia in Greece since 1943. Proc. Scientific Soc. "Evangelismos" Hospital Athens 35: 20, 1944.

———: Mastoplasia in the male (gynecomastia). Proc. Greek Surgic. Soc. 25: 224, 1947.

———: Malnutrition and gynecomastia. Proc. Am. Soc. Clin. Path. October 1949.

Problems for Consideration in Demographical Studies of Cancer. A. LACASSAGNE, *Institut du Radium de l'Université de Paris, Paris, France.*

Abstract. Dr. Lacassagne pointed to the difficulties involved in obtaining accurate statistical data on cancer, and in this connection he pointed to the part played by age and latency period. He also reviewed the information which had been obtained by other means with regard to the distribution of tumors on site and tissues.

Secondly, he mentioned the multiplicity of factors presumably involved, the variation in the histological picture and in susceptibility of the host of the tumor, which all tend to create difficulties in our research.

As the closest subjects for examination, Dr. Lacassagne suggested primary cancer of the liver in various races, and cervical cancer among Jewesses. A closer collaboration with veterinary surgeons might contribute to our knowledge of the etiology of cancer. For instance, inspired by the extraordinary frequency of liver changes among swine in Morocco, Ninard and Hintermann reached interesting conclusions on the transformation of liver cells into cancer cells.

Also, comparative studies of different races living in the same surroundings will be a practical way of approach to the problems confronting us. Special studies or customs in certain cultures may also be fruitful.

Suggestions of Problems for the Consideration of Cancer Demography and of Experimental Cancer Research. J. ENGELBRETH-HOLM, *The University Institute of Pathological Anatomy, Copenhagen, Denmark.*

Abstract. In order to obtain fully reliable results in research work on a demographical basis, the institution of absolute, uniform, international methods of registration would be vital. Likewise, the application of a uniform nomenclature is of fundamental importance.

The achievement of these and similar objects is however utopian, for the time being, and if we lived to see the fulfillment of these theoretical demands, we could almost at once leave further research in the hands of mechanical calculators.

In experimental cancer research we have for many years worked without exact methods, but nobody has doubted the justification of the work for this reason, and we are now approaching, though slowly, the application of more accurate means.

Suggestions Regarding Problems to be Taken into Consideration in a Demographic Study of Cancer. CHARLES OBERLING, *Professor of Carcinology at Faculté de Mèdicine, Paris, France.*

Abstract. The extraordinary variability of the different forms of cancer according to the countries is a fact which has for a long time struck observers, but until now has not received the attention that it deserves. First of all, there is the question of obtaining exact information in this field; impressions must be replaced by data that are based on figures. It is said that pulmonary cancer is not found in Iceland and Korea, that cancer of the pancreas is one of the most frequent in Finland, that gastric cancer is much rarer in England than on the continent, *etc.* . . . , but all this needs to be backed up with statistics. Among the few established facts seems to be the extraordinary frequency of hepatic cancer in the Dutch East Indies, of cancer of the penis in the populations of Indochina, and of sarcoma in the populations of North Africa. A very detailed investigation must be made throughout the whole world to establish the frequency of the different forms of cancer.

Moreover, it is extremely important that this statistical inquiry be supplemented by a sociological study of the mode of living, the habitual intoxications, and the diet of the various populations.

Innumerable problems capable of supplying very interesting information on the etiology of cancer thus await solution.

Reference

DUNGAL, N.: Lung carcinoma in Iceland. Lancet 259: 245, 1950.

Forms of Cancer in Man Suitable for Investigation. E. L. KENNAWAY, *St. Bartholomew's Hospital, London, England.*

Abstract. The material brought forward at this Congress has given some very definite and promising indications for inquiry. These subjects may be divided into the more general, and the more special problems.

I. *General Problems.*

The whole subject of nutrition, in the widest sense, in relation to carcinogenesis, requires examination. Thus, the response of any tissue to a carcinogen depends upon both the more recent and the less recent quantitative and qualitative characters of the diet. This study requires both experimental work on animals and observations upon human populations living under a great variety of conditions. We have no data about two fundamental factors in the occurrence of cancer in man:

A. The minimum effective dose of any carcinogen and the relation of dose to duration of exposure.

B. The possible summation of effect of two carcinogens, *e. g.*, benzpyrene and arsenic in the dust of towns, or radioactivity and arsenic in the air of a mine.

II. *Special Problems.*

The following may be suggested:

A. *The high incidence of primary cancer of the liver in the Javanese and in the Bantu of South Africa*, who are as different physically as any two races. This in all probability is a dietetic problem. The field has been narrowed somewhat by Dr. Khanolkar, who tells us that in the Bombay area, where there is plenty of malnutrition, primary liver cancer is very much less frequent than it is in Java and South Africa.

B. *The contrast in the incidence of stomach cancer in Indonesia* (*High in Chinese, very rare in Javanese*).—This may also be a dietetic problem including in that term methods of cooking. Dr. Berman has stated that cancer of the stomach is very rare in the South African, also among Bantu mine workers in whom primary liver cancer is very common. Is there any association between the high incidence of cancer of the liver and the low incidence of cancer of the stomach? A study is required of cancer of the stomach in relation to diet and methods of cooking, with especial reference to the temperatures to which food is heated. In this respect there are considerable ethnological differences. One can distinguish at least three types:

Type 1—*Multiple methods of heating*, including roasting, baking, grilling, and frying. These methods prevail in most urbanized and industrialized countries.

Type 2—*Slow cooking by hot ashes and hot stones.*—These methods are used especially in the South Pacific and Southeast Asia. The bulk of the food is not heated to the temperatures reached in Type 1.

Type 3—*Boiling methods*, which produce chiefly cereal porridges that have not been heated above the temperature of boiling water. The cookery of the Bantu in South Africa appears to be largely of this type. A state of civilization is required in which metal or clay pots are available that will stand heating; more primitive peoples may use roasting methods similar to those of Type 1. The Esquimo method of heating over seal-oil lamps is of this class.

Data are required for

a. Diet, and especially the use of highly spiced foods, which is customary in Java and in West Africa, where this form of cancer is much more common than in Europe (Denoix) but not in South Africa. The recent experimental work of C. Hoch Ligeti on chilis from Java (V^{e} Congres International du Cancer, Paris, 1950) is of interest in this respect.

b. Dietary deficiencies.

c. Cereals used (maize, millet, or others).

C. *The association of cancer of the lung with smoking*, especially of cigarettes, found by Doll and Bradford Hill in England, and by Wynder and Graham in the U. S. A. We must consider whether tobacco smoke (a) contains a carcinogen, or (b) increases the liability of the bronchi to be acted upon by other agents, *e. g.*, the smoke of towns. We must inquire, in selected countries, about (a) the incidence of lung cancer in the

two sexes separately; (b) the kind of tobacco smoked, whether arsenic-rich (American) or arsenic-poor (Turkish); (c) the form in which it is smoked (cigarette, cigar, pipe). Data are required about any change in these respects in the last 25 years. In any such inquiry, cancer of the larynx, in both sexes, should be included, especially in view of the results from North Africa.

D. *Cancer of the cervix uteri in women under different sexual conditions*, and especially in Jewish women.

Such an inquiry is being undertaken by Dr. Symeonidis in New York, and in Israel, and by Dr. Doll in London, and they have independently devised very similar questionnaires for this purpose.

1. The question, whether the abstention from intercourse after menstruation enjoined by Jewish law is of any importance, could be settled very soon if reliable information about their practice in this respect could be obtained from the few Jewish women who develop cancer of the cervix.

2. The relation of circumcision to cancer of the cervix is also open to investigation. Data are required about the proportion of circumcised husbands of women who do, and of those who do not, develop cancer of the cervix.

Discussion

DR. CLEMMESEN: I do not share Dr. Lacassagne's fear of the large number of problems mentioned, in view of the numerous institutes represented, but I admit that the multiplicity of factors possibly involved in the carcinogenesis of a single type of cancer can scare me. The more unknown factors, the more equations necessary to solve them. But it is just on this point that we are looking for the active support from experimental workers.

As possible areas for interesting studies I would mention both South America and Japan.

DR. STEWART: As to Dr. Lacassagne's suggestion on cancer research in domestic animals, I think the cattle being handled in some of the large abattoirs would provide material for studies (Jackson 1936).

DR. ENGELBRETH-HOLM: Nevertheless, I believe that geographical investigations on animals will on the whole be a disappointment, because they are killed before they reach the cancer age. In my experience there are only three results: leukemia in cattle, sarcomas in hares, and papillomas in eels near Peenemunde.

MME. MUSSINI-MONTPELLIER: I think I can state that cancer in the digestive tract is very uncommon in domestic animals.

DR. MAISIN: Yet we have Schwannosarcomas in mules. I would be interested to know about the interrelation of bilharziasis in the intestine and the bladder in relation to cancer.

MME. MUSSINI-MONTPELLIER: In South Tunisia we find many cases of bilharziasis, and many cases of cancer in the bladder.

DR. KHANOLKAR: It is my experience from India that the urinary type of bilharzia gives rise to vesical cancer, but the intestinal type does not give rise to rectal cancer.

It is also necessary to bring to the notice of the conference once again the marked difference in the prevalence of the cancer of the base of the tongue in some communities in India. Whereas in western countries cancer of the base of the tongue constitutes only 25 percent of tongue cancer, in certain communities in India it is the commonest type of cancer. The reason for this frequency deserves investigation. Cancer of the base of the tongue and the tonsils is roughly 80 percent of all oral cancer in the males of these communities.

References

DOLL, R., and HILL, A. B.: Cigarettes and cancer. Brit. M. J. No. 4682: 767, 1950.

JACKSON, C.: The Incidence and Pathology of Tumors of Domestic Animals in South Africa. Pretoria, 1936.

KENNAWAY, E. L.: Cancer of the liver in the Negro in Africa and in America. Cancer Research 4: 571–577, 1944.

———: Cancer of the penis and circumcision in relation to the incubation period of cancer. Brit. J. Cancer 1: 335–344, 1947.

———: The racial and social incidence of cancer of the uterus. Brit. J. Cancer 2: 177–212, 1948.

———: The data relating to cancer in the publications of The General Register Office. Brit. J. Cancer 4: 158, 1950.

KENNAWAY, E. L., and KENNAWAY, N. M.: Some factors affecting carcinogenesis. Acta Unio Internat. contra Cancrum 2: 101–135, 1937.

———: A further study of the incidence of cancer of the lung and larynx. Brit. J. Cancer 1: 260–298, 1947.

WYNDER, E. L., and GRAHAM, E. A.: Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma. J. A. M. A. 143: 329–338, 1950.

The Application of Pathology to the Field of Cancer Demography. A. SYMEONIDIS, *Geographic Pathology Unit, Pathology Section, National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md., U. S. A.*

Abstract. Cancer in human beings has been relatively neglected, although it offers excellent material for studies fundamental to many cancer problems, including the problem of carcinogenesis. Geographic pathology is a most promising field for the investigation of human material.

Histopathological studies on types of cancer made in close correlation with statistical surveys may open unexplored fields in cancer research. Special intensive investigations in selected geographical areas or ethnical groups that have a significant variation in the anatomical location and the histological types of recognized cancer will probably lead more directly to our ultimate objective, which is the recognition of etiological factors. Other diseases associated with cancer, and lesions that are suspected to be precursors of neoplasia, are also of importance. Comparative histopathological studies of particular types of cancer and a uniform review of material from different geographical areas or ethnical groups will provide reliable foundation for further studies. The importance of differences in customs, nutrition, and endocrinological and other factors can then be estimated. Such studies on restricted material are feasible on a world-wide scale at the present time, and these studies could serve as models for future extensive studies covering the whole field of human cancer.

The following base lines have been drawn for the organization of the unit of geographical pathology and the methods to be used in our investigative program:

1. Geographical pathology of cancer is a research branch for studies of carcinogenesis in humans.

2. The nature of this kind of research work must be based on a carefully planned and close cooperation among scientists of many disciplines—as pathologists, statisticians, nutritionists, and so on—in different geographical areas and in a kind of teamwork that must be based on a common, concrete plan.

3. Pathological anatomy will play a very important role, especially for the ground work and the initiating period of these investigations.

4. Comparative pathological anatomical study of material from biopsies and autopsies of selected special subjects from critical geographical areas and ethnical groups is, in my opinion, the method of selection for geographical cancer research. I should like to call it "comparative human pathology."

5. Distribution and exchange of pathological material among the participants of the studies in different geographical areas is feasible and not expensive.

6. A close cooperation between the participants during the progress of studies, followed by a final meeting for drawing the final conclusions, will secure valuable results.

7. Experimental investigations will take over promising problems that arise during these studies.

The unit of geographical pathology recently established at the National Cancer Institute has made preliminary studies on two types of cancer which, according to our existing knowledge, show a striking difference of incidence in different geographical areas and ethnical groups. These are 1) cancer of the cervix, which has a low incidence in Jewish women and a high incidence in non-Jewish women, and 2) primary cancer of the liver, which has a low incidence in some areas of Europe and the United States and a high incidence in other areas such as Africa and Greece.

These studies will be carried out on a collaborative basis with pathologists and cancer endemiologists. The subjects and the background of these selected statistical and pathological methods that have been developed during this preliminary phase of our studies have been presented.

The Representation of Results in the Study of Geographical Pathology of Cancer. J. M. MAY, *The American Cancer Society, New York, N. Y., U. S. A.*

Abstract. (This paper was presented by Dr. Harold F. Dorn, who demonstrated a series of cartographic methods worked out by Dr. May for the representation of the various kinds of information, necessary to maps, indicating the occurrence of cancer in a given area.)

The Administrative Aspects of Organizing a Unit Devoted to the Study of Geographical Pathology of Cancer. HAROLD L. STEWART, *Pathology Section, National Cancer Institute, Bethesda, Md., U. S. A.*

Abstract. We may not for a long period obtain valid comprehensive statistics on cancer incidence, but we can immediately begin to study the distribution of anatomical and histopathological types of cancer, precancerous conditions, and types of cancer. It would appear reasonable to expect that different etiological factors might express themselves in terms of such differences.

For this purpose the Unit of Geographical Pathology of Cancer was established at the National Cancer Institute.

Dr. Stewart gave a full description of the administrative aspects and problems of such a Unit.

Practical Suggestions for a Program of Study of Cancer from the Point of View of its Distribution in Various Population Groups. MAURICE B. VISSCHER, *Department of Physiology, University of Minnesota, Minneapolis, Minn., U. S. A.*

Abstract. Dr. Visscher outlined the wider organizational issues involved in the establishment of an international collaboration in the field of geographical pathology and demography of cancer.

The discussion following these papers resulted in the recommendations passed.

Practical Suggestions. B. S. PLATT, *Human Nutrition Research Unit, Medical Research Council, England.*

Abstract. In conformity with the desire for practical suggestions expressed in the invitations for the conference Professor Platt made the following proposals:

Propose trial method of running a Regional Conference or Review by arranging in 1 to 1½ years' time a series of meetings, discussions, and demonstrations in representative parts of a selected region.

Select the region in relation to a problem, *e. g.*, primary carcinoma of the liver in Africa.

Include scientists occupied with closely related aspects of liver disease, *e. g.*, nutritional liver disease and diseases, such as malaria, causing liver damage.

Provide for one or more members to attend two or more meetings in each of the representative centers in North, South, East, West, and Central Africa.

Secure co-operation of various governments, *i. e.*, of France, Belgium, Portugal, South Africa, Great Britain, and Egypt.

Include one representative of areas of possible future regions in which conferences might be held, *e. g.*, India, Far East, and South America.

Begin early to acquaint workers in medicine, pathology, and nutrition in the various *areas* in the region of the proposed meetings, so that they may contribute to the proceedings in their respective *areas*.

Make use of the needs and experience of the established unit or units for "endemiological research" as a guide to the scope and aims of the proposed regional review—at the time of the review and future activities.

○